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LOCALIZED ARTERIOSCLEROTIC LESIONS INDUCED IN THE AORTA OF THE JUVENILE RABBIT BY FREEZING

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CHICAGO

JOSUÉ [1] was the first to report, in 1903, that arteriosclerosis had been produced in rabbits by injecting epinephrine hydrochloride intravenously. Since that time, numerous investigators have attempted to reproduce this work, using epinephrine hydrochloride and other substances with varying success. Whenever lesions were produced in the aortic wall, they were similar to a type of human peripheral arteriosclerosis characterized principally by degeneration of musculoelastic tissue of the media, medial deposition of calcium and proliferation of the intima.

Only a few authors have mentioned the reparative changes which followed the development of these lesions. Scheidemandel [2] expressed the belief that the calcium deposited in the media was resorbed in rabbits that were kept alive long enough. Pearce and Stanton [3] mentioned cellular infiltration around calcium deposits and repair of a few degenerative lesions of the aortas of rabbits. Bailey,[4] having produced similar lesions in rabbits by injecting diphtheria toxin, stated that regeneration of the media and compensatory intimal hyperplasia might occur if the animals could be kept alive. Furthermore, Erb,[5] Hesse [6] and Cowdry [7] have described instances in which new smooth muscle cells and elastic fibers have appeared during repair of damaged vessels of rabbits. Apparently, a careful analysis of the repair of the aortic tissues following injury

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1. Josué, M. O.: Compt. rend. Soc. de biol. **55**:1374, 1903.
2. Scheidemandel, E.: Virchows Arch. f. path. Anat. **181**:363, 1905.
3. Pearce, R. M., and Stanton, E. McD.: Proc. Soc. Exper. Biol. & Med. **2**:77, 1904.
4. Bailey, C. H.: J. Exper. Med. **25**:109, 1917.
5. Erb, W.: Arch. f. exper. Path. u. Pharmakol. **83**:173, 1905.
6. Hesse, M.: Virchows Arch. f. path. Anat. **249**:437, 1924.
7. Cowdry, E. V.: Arteriosclerosis: A Survey of the Problem, New York, The Macmillan Company, 1933, pp. 273-274.

has not been made because no controlled means was available for reproducing a lesion in a specific location in an identical manner in successive animals.

The studies reported here were designed to produce identical arteriosclerotic lesions in the aortas of successive young rabbits and to evaluate the roles of degeneration and regeneration in the arteriosclerotic process over a period of six months.

METHODS

Male New Zealand white rabbits, 3 months of age and weighing between 2 and 2.5 kilograms, were used. Each rabbit was anesthetized with ether. A midline abdominal incision was made, and the abdominal aorta was dissected free from

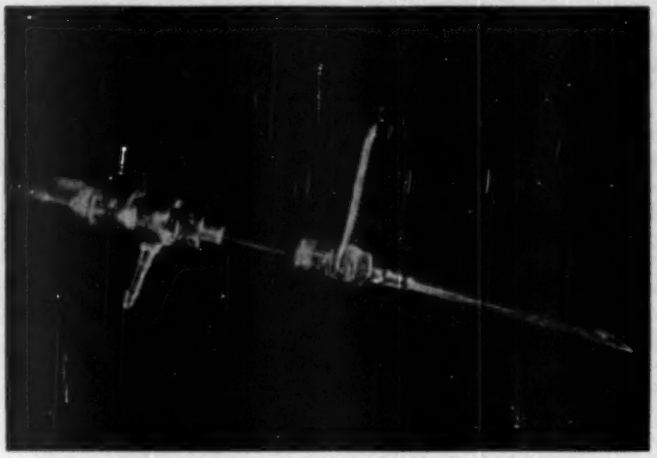

Fig. 1.—The needle used for freezing the aorta.

the retroperitoneal tissues in six places. The freezing instrument used for controlled quantitative production of aortic lesions was composed of two needles of 16 and 19 gage.[8] The larger needle was closed at its distal end by solder. At its proximal end, a Luer-Lok sidearm fitting was soldered to the base of the smaller needle, which was fitted inside the larger, and shortened so that its distal end did not reach the sealed end of the larger needle (fig. 1). Carbon dioxide, maintained in a standard commercial cylinder at 800 to 1,100 pounds per square inch (about 56 to 75.5 kilograms per square centimeter), was allowed to pass through the smaller needle into the closed chamber formed by the larger needle and out through a sidearm exhaust. Lesions were made by passing the needle transversely behind the aorta and elevating it. When the blood flow was completely obstructed by tension produced by elevation of the aorta, the gas was allowed to pass through

8. Hass, G. M., and Taylor, C. B.: Arch. Path. 45:563, 1948.

the needle for the duration of the period selected for freezing. Freezing was almost instantaneous, not only of the portion of the vessel in contact with the needle but of the rest of the circumference as well. Warm saline solution was then dropped on the needle at the end of the time interval selected for freezing. This made it easy to release the needle from the frozen aortic wall promptly. The aorta of each rabbit was frozen in six places, approximately 0.5 cm. apart.

The six locations in the aorta were frozen for 10, 15, 40, 20, 30 and 60 seconds, respectively, being placed in that order down the aorta in the even-numbered rabbits, and in the reverse order in the odd. No lesion was made above the origin of the renal arteries or below the origin of the common iliac arteries. Each lesion was marked for future identification with a black silk ligature tied in the adjacent muscle of the posterior abdominal wall. The surgical wound in the abdominal wall was closed with silk. Each rabbit was allowed to live for the number of weeks selected and then was killed by an intravenous injection of thiopental sodium (pentothal sodium® N. N. R.). The abdominal aorta was dissected free from the retroperitoneal tissues. The lesions of the aorta were identified by the silk sutures in adjacent muscle. The aorta was pinned to a wooden splint. The site of each lesion was indicated on the splint and then the aorta was resected.

Each aorta was fixed in Zenker's solution, and after fixation each lesion was resected and individually embedded in paraffin. Serial sections, 10 microns in thickness, were made through each lesion. Every fifteenth section was mounted for microscopic study. Most lesions were cut so as to show the circumference of the aorta in cross section. Longitudinal sections were made in a few instances. The sections were stained with hematoxylin and eosin and, when desired, with Weigert's stain for elastic tissue or Mallory's phosphotungstic acid-hematoxylin connective tissue stain for definition of smooth muscle. Normal control sections were taken proximal and distal to the segment of aorta containing the lesion. The section showing maximum change as compared with the control sections was selected for description and appraisal. Criteria were established for the regenerative trends found in all the lesions studied, and the section showing the maximum changes within each lesion was graded accordingly.

Lesions were produced in 26 rabbits, which were allowed to live for varying intervals of time ranging from one to twenty-four weeks. In most instances, at least 2 rabbits were retained for each weekly interval. Serial sections of 142 lesions were prepared for microscopic study.

RESULTS

The tissue reaction in segments of aorta injured by freezing showed a constantly changing pattern which was independent of the duration of freezing (10 to 60 seconds). It was possible to divide this reaction into several distinct processes which could be followed throughout the periods of six months. Many processes were of limited duration and terminated in the most extensive disturbance of normal structure. These processes were arbitrarily defined as *degenerative*; they culminated in a pattern of peripheral sclerosis similar to that described by Mönckeberg.[9] Others were of prolonged duration and tended to reproduce normal structure

9. Mönckeberg, J. G.: Virchows Arch. f. path. Anat. **171**:141, 1903; Klin. Wchnschr. **3**:1473, 1924.

of the aortic wall. These were arbitrarily defined as *regenerative;* they culminated in repair of the lesion. The microscopic sequences in each process are described in the following paragraphs.

DEGENERATIVE PROCESSES

1. Function of smooth muscle was immediately impaired because freezing was instantly followed by local aneurysmal dilatation of the aorta. Furthermore, disintegration of intramedial smooth muscle cells was the earliest significant morphologic change. This was conspicuous at the end of the first week. It appeared first in small strips, which increased in size and number until, after two weeks, the majority of the lesions showed complete degeneration of smooth muscle in all serial sections (fig. 2 *A* and *B*). During this process, smooth muscle cells became swollen and refractory to stains, and their nuclei became irregular and pyknotic. As the cells disintegrated, nuclear debris became prominent and persisted longer than traces of cytoplasmic structure.

2. Degeneration of the elastic lamellas became conspicuous after degeneration of smooth muscle had proceeded to an advanced stage (figs. 2 to 5). By the end of the second week the lamellas had become fused and swollen. Individual elastic fibers lost their normal wavy contour, became linear and seemed to be fused into fragile amorphous blocks, which appeared to be broken in many places. All lamellas except the internal and external elastic membranes underwent this type of degeneration. These two structures usually showed no significant changes apart from occasional discontinuity.

3. Calcification of the media usually appeared in the third week, although in one instance it was present as early as the first week (fig. 2 *A*). Following loss of smooth muscle and swelling of elastic fibrils, small, deeply stained granules of calcium salts began to appear along the margins of elastic lamellas apparently being deposited in the interlamellar matrix (fig. 2 *B*). These granules increased in number until, by the fourth or fifth week, all or part of the media within the lesion was diffusely calcified (fig. 4). Yet, even after this process had become prominent, it was still often possible to distinguish outlines of elastic lamellas within the dark-staining plaque of calcium deposits.

4. An inflammatory response was present in many lesions for as long as two to three weeks (fig. 2 *A*). This took the form of scattered aggregations of neutrophilic cells of the granulocytic series and lymphocytes in the adventitia. The media and the intima were not involved. Control lesions in which the aorta was dissected from retroperitoneal tissues without subsequent freezing showed the same occasional inflammatory

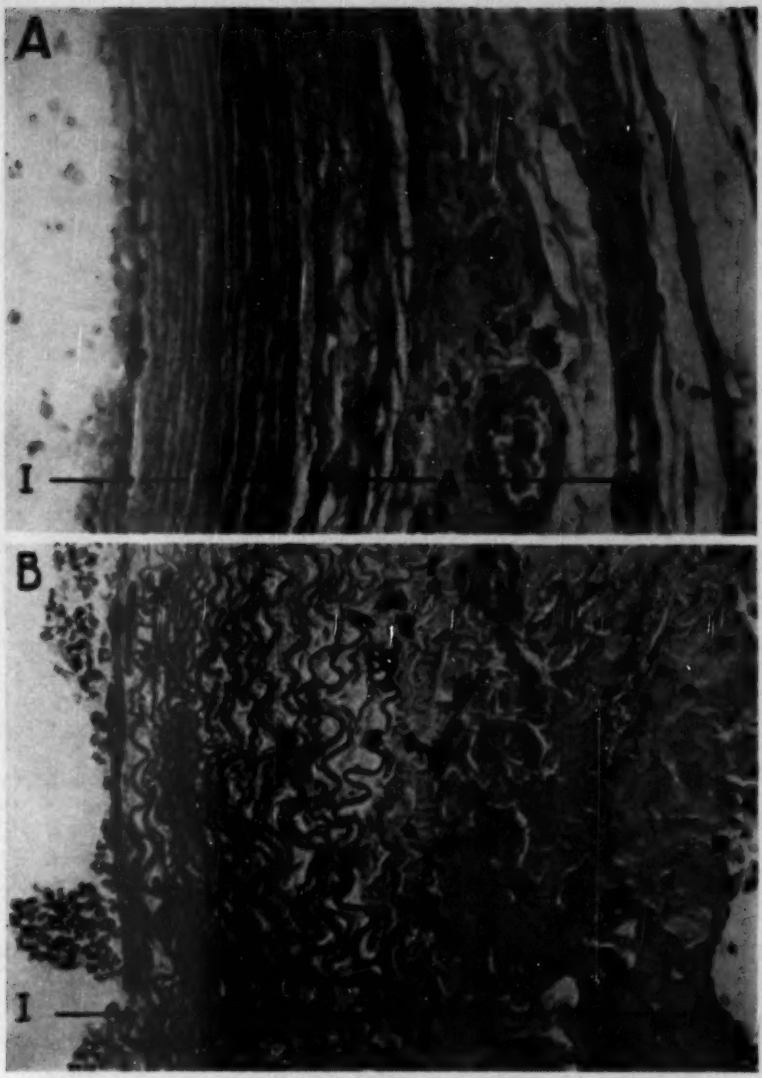

Fig. 2.—*A*, cross section of an aortic wall one week after freezing. Intimal endothelium (*I*) has either persisted or regenerated. Smooth muscle cells of the media (*M*) have disintegrated. Granules of calcium have appeared in the matrix between elastic lamellas of the media. The elastic lamellas are straight. Rare inflammatory cells are in the adventitia (*A*).

B, cross section of an aortic wall two weeks after freezing. There is early proliferation of cells in the intima (*I*). Elastic lamellas of the media (*M*) show discontinuities and variability in thickness, spacing and undulation. Remnants of degenerated smooth muscle cells have largely disappeared. Granules of calcium are barely distinguishable in the interlamellar matrix of the media. Collagenous fibrils of the adventitia are increased and fused into homogeneous bundles surrounding fibroblasts and mononuclear inflammatory cells.

reponse without any degenerative change in the media or the intima. At the same time, many lesions showed no evidence of inflammation, and inflammatory cells were rare in all lesions older than five weeks.

5. Fibrosis of the adventitia was present in most lesions after from one to twenty-four weeks. The collagen fibrils became fused into pale-staining hyaline bundles surrounding the aorta (figs. 2 and 4 *B*).

TABLE 1.—*A Summary of the Relative Magnitudes of Degenerative Changes in Aortic Lesions of Increasing Age*

Rabbit	Age of Lesions	Loss of Smooth Muscle	Degeneration of the Elastic Lamellae	Medial Calcification	Perivascular Inflammation	Adventitial Fibrosis	Bone
22	6 days	+	+	0	+	++	0
4	1 week	++	0	+	+	+++	0
23	1 week	+++	+++	0	++	++	0
30	8 days	++	+	0	+	++	0
32	2 weeks	+++	+++	0	+	+++	0
33	2 weeks	+++	++	0	0	+++	0
7	3 weeks	+	+	0	+	+++	0
60	3 weeks	++	++	+	0	+++	0
0	3 weeks 1 day	++	++	+	++	+++	0
12	4 weeks	+++	+++	+++	0	+++	0
29	4 weeks	++	+	0	0	+++	0
37	4 weeks	++	+++	+	0	++	0
17	5 weeks	++	++	+++	+	+++	0
9	5 weeks	++	++	++	+++	+++	0
3	6 weeks	+	+	0	+	+++	0
27	6 weeks	++	++	++	0	++	0
43	6½ weeks	++	++	+++	0	+++	0
20	8 weeks	++	++	+++	0	+++	0
26	8 weeks	++	++	+++	++	+++	0
25	10 weeks	++	++	++	0	+++	0
16	10 weeks	++	++	++	0	++	0
11	11 weeks	+++	++	++	0	++	0
15	13 weeks	+	+	+	0	++	0
30	17½ weeks	++	+	++	0	++	Trace
35	20 weeks	++	+	++	0	++	Trace
31	24 weeks	++	++	++	0	+	0

6. Primitive osteoblasts, osteoid tissue and bone were found in small, isolated calcified foci in 2 of the 142 lesions studied. These lesions were seventeen and a half and twenty weeks old. Ossification occurred principally in the adventitia. In both cases adjacent intramedial deposits of calcium were undergoing resorption.

Table 1 is a summary of our attempts to grade degenerative processes in a semiquantitative way. This table shows that there was consistent evidence of degeneration of smooth muscle and elastic tissue in the media. Medial calcification was fairly extensive in most lesions after the fourth week. The inflammatory reaction was inconstant and appeared to have little significance. Furthermore, it bore no spatial

relation to changes in the media and the intima. Bone formation was rare and occurred only after many weeks had passed. Finally, control sections taken from the aorta above and below the lesions showed no pathologic changes.

REGENERATIVE PROCESSES

1. The proliferation of the intima was classified as a process of repair inasmuch as it furnished the framework within which new vascular structure was to differentiate. The earliest evidence of intimal proliferation was found in lesions at the end of two weeks (fig. 2 *B*). First a thin reticular strip of tissue appeared along one side of the dilated lumen. This gradually proliferated around the lumen and advanced centrally toward the axis of the vessel, compensating for the dilation caused by relaxation of the vessel following freezing and recreating a lumen of normal size (fig. 3 *A*). Where dilation of the aorta was greatest, namely, at the point of maximum damage, the proliferation of the intima often was equal to the thickness of the damaged aortic wall. Intimal proliferation was practically complete at five weeks and provided a structural matrix for regeneration of a new media (fig. 4 *A*).

2. Proliferation of elastic tissue within the intima is shown in figures 3 *B* and 5 *B*, photomicrographs of Weigert-stained sections from lesions five and twenty weeks old. Thin, scattered elastic fibrils appeared within the matrix created by the proliferation of the intima in lesions three weeks of age. These fibrils gradually increased in number and width until the new elastic structure became extremely dense. Although the old internal elastic membrane occasionally split, new elastic fibrils did not originate from undamaged elastic tissue but developed in the matrix of proliferated intima. At the end of twenty weeks, even though elastic regeneration was still incomplete, new fibrils had about the same dimensions as the original medial elastic lamellas of the aortic wall (fig. 5 *B*).

3. Proliferation of smooth muscle appeared within proliferating intima at the end of two to three weeks in the form of large, pale cells containing pink-staining cytoplasm and vesicular nuclei (fig. 3 *A*). These immature cells gradually matured and attained the spindle-like form of smooth muscle cells, similar to those found in undamaged media (fig. 4 *A*). These cells increased in number until, after the sixth week, the structure of smooth muscle within the proliferated intima became almost identical with that of normal media (fig. 4 *B*).

4. In all lesions older than four weeks a new internal elastic membrane could be distinguished beneath the endothelium which overlay the proliferated intima (fig. 3 *B* and 5 *A*). The elastic fibrils which had

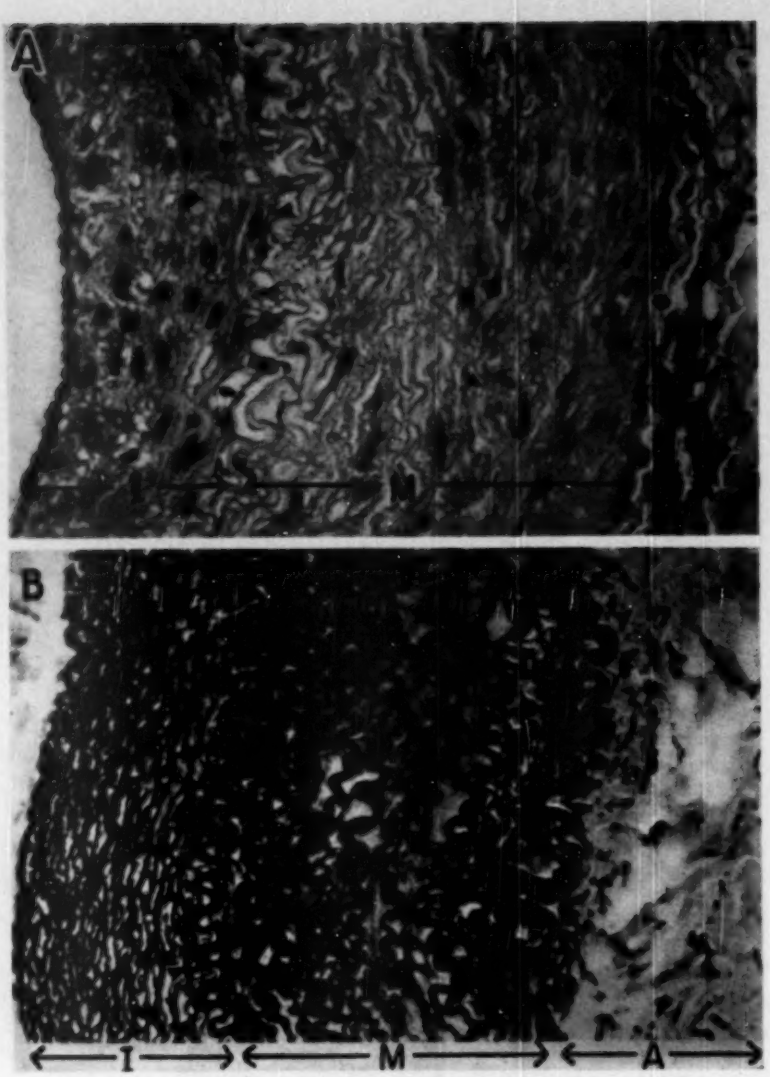

Fig. 3.—*A*, cross section of an aortic wall three weeks after freezing. There is evidence of active proliferation of intima (*I*) with numerous elongated cells, differentiating into smooth muscle cells, and an abundant intercellular fibrillar matrix. The media (*M*) shows irregular elastic lamellas and loss of smooth muscle. Fibroblasts, some of which eventually differentiate into smooth muscle cells, have grown between remnants of elastic lamellas in the outer one half of the media. Wavy outlines of persistent internal and external elastic membranes are detectable. The adventitia (*A*) resembles that of figure 2 *B*.

B, elastic tissue (Weigert stain) of a cross section of an aortic wall five weeks after freezing. The proliferated intima (*I*) contains many thick fibrils which have become oriented to resemble thin medial elastic lamellas. Elastic lamellas of the media (M) apart from the internal and external elastic membranes are discontinuous, irregular and distorted. Elastic tissue of the adventitia (*A*) is normal.

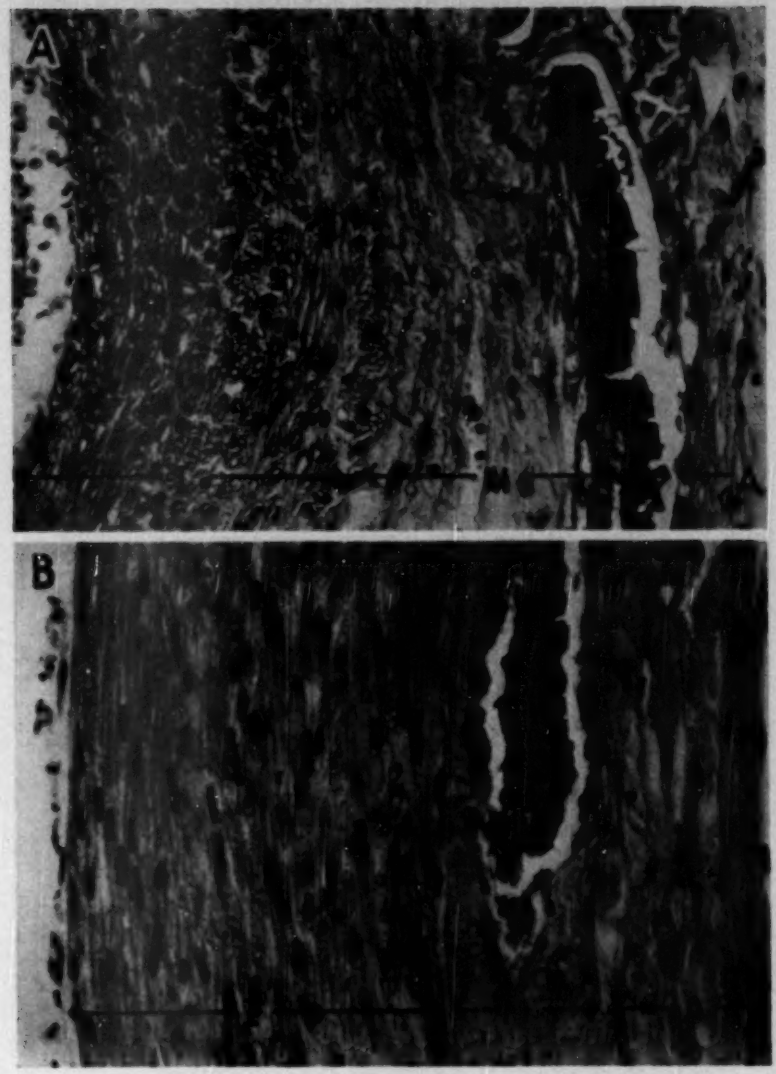

Fig. 4.—*A*, cross section of an aortic wall eight weeks after freezing. The regenerated intima (*I*) closely resembles the media of a normal aorta. Most cells have the morphologic aspects, distribution and staining reactions of smooth muscle cells. The matrix between these cells is composed principally of elastic tissue. The damaged media (*M*) contains scattered fibrocytes, collagenous fibrils and degenerated elastic lamellas in its inner half. The outer half of the media (*M*) is a homogeneous mass of fragmented calcified tissue. The calcium deposits are partly surrounded by macrophages and show evidence of early resorption.

B, cross section of an aortic wall twelve weeks after freezing. The intima (*I*) at this stage is as thick as the original undamaged aortic media. It is composed principally of parallel smooth muscle cells lying between thick lamellas of elastic tissue. The badly damaged media (*M*) is largely occupied by masses of calcium, which are surrounded by macrophages and dense fibrous tissue. The adventitia (*A*) is composed of dense bundles of fused collagenous fibrils.

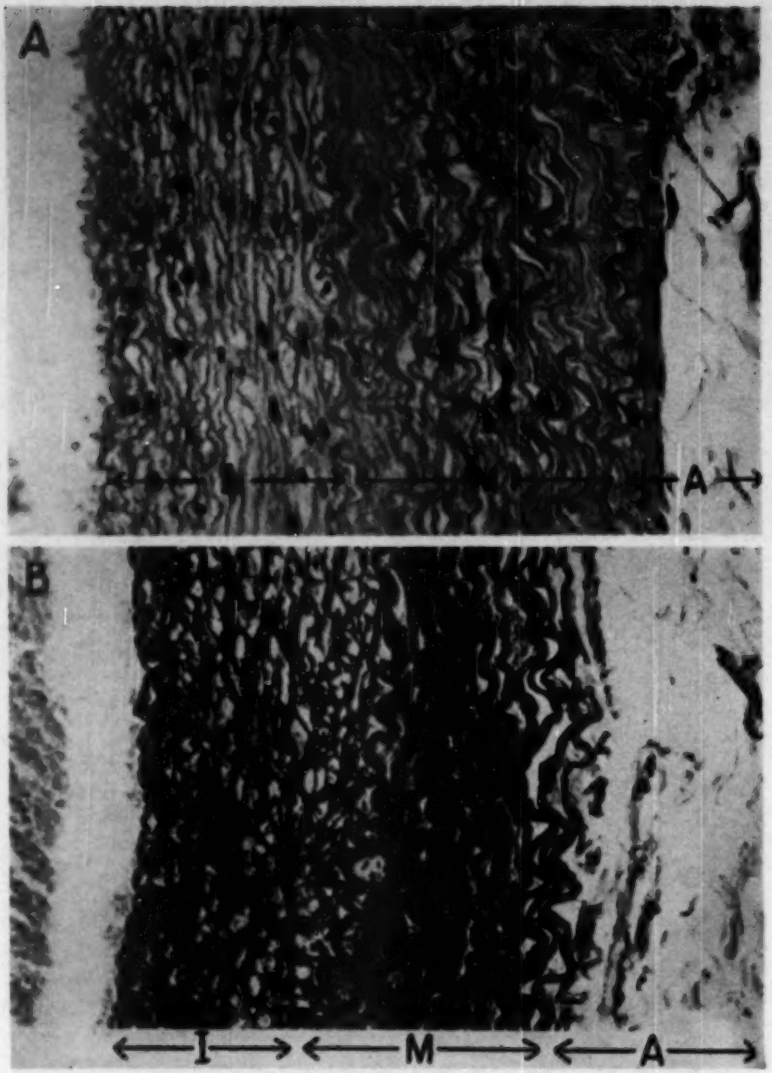

Fig. 5.—*A*, cross section of an aortic wall twenty weeks after freezing. Damage to the media (*M*) was not severe. Proliferation of intima (*I*) was moderate with a less conspicuous maturation of smooth muscle cells and elastic tissue. Elastic lamellas of the damaged media have persisted. Smooth muscle cells have degenerated, though a few immature cells resembling smooth muscle cells may be seen between the persistent elastic lamellas. There are no deposits of calcium. The adventitia (*A*) is normal.

B, elastic tissue (Weigert stain) of a cross section of the aortic wall shown in *A*. The lesion was twenty weeks old. There is good preservation of elastic tissue in the media (*M*), indicating that the original damage was slight. Nevertheless, there is conspicuous formation of elastic tissue in the framework of proliferated intima (*I*). A new internal elastic membrane, adjacent to the lumen of the vessel, can be compared with the persistent original internal and external elastic lamellas. Few elastic fibrils are found in the adventitia (*A*).

developed within the thickened intima increased in number in the subendothelial zone and gradually fused together to form a well defined membrane (fig. 5 *B*). In the earlier stages this membrane stained purple with hematoxylin and eosin. In only a few sections from the oldest lesions did it have the pink color of mature elastic tissue.

5. The smooth muscle within the old media began to proliferate during the second week. However, in most instances the old media was so badly damaged by freezing that few smooth muscle cells were left, and regeneration was only partial and scattered (fig. 4 *A*). Figure 4 *B* shows a section in which the old wall is largely composed of fibrous tissue, without evidence of regeneration of smooth muscle cells. On the other hand, partial regeneration of smooth muscle cells is demonstated in figure 5 *A*.

6. After four weeks, most lesions showed that calcium deposited in the media was being slowly resorbed. The process of resorption was characterized by a gradual loss of definition and serration of margins of the calcified blocks, by local giant cell reaction and by accumulation of deeply stained granular debris around the periphery of the calcified areas (fig. 4). Furthermore, the older the lesion, the smaller, in general, were the calcium plaques. In the oldest lesions the calcium deposits were often restricted to small islands of deeply stained material found only at margins of lesions. Resorption of calcium was relatively inconstant, some lesions showing extreme evidence of this process and others of the same age showing little. Furthermore, it progressed at a much slower rate than other processes of regeneration.

A semiquantitative description of the activity of repair was possible in the first four reparative processes because valid criteria could be formulated. By these, the section showing the maximum change in each lesion was graded.

CRITERIA FOR GRADING OF REGENERATIVE PROCESSES

Intimal Proliferation.

Grade 0. No intimal proliferation in any part of the lesion.

Grade 1. Localized intimal proliferation, not extending around more than one half of the circumference of the maximal lesion or exceeding a width of 3 to 4 cells.

Grade 2. Intimal proliferation extending around not more than one half of the circumference of the maximal lesion or exceeding the old wall in width, yet wider than the thin strip qualified under grade 1.

Grade 3. Intimal proliferation extending around more than one half of the circumference and not equaling the width of the old wall at any point.

Grade 4. Intimal proliferation extending around the circumference of the maximum lesion and equaling the old wall in width.

Proliferation of Elastic Tissue Within the Intimal Proliferation.

Grade 0. No proliferation of elastic tissue within the intimal proliferation.

Grade 1. Proliferation composed of elastic lamellas of extreme thinness.

Grade 2. More than 10 to 15 elastic lamellas in the proliferated intima with an elastic structure of low density.

Grade 3. Elastic structure of high density with large numbers of lamellas, the majority of which did not equal the old lamellas in width.

Grade 4. Elastic proliferation of a density commensurate with that of the original aortic media, with elastic lamellas of the same average width as those in the original media.

Proliferation of Smooth Muscle Cells Within the Intimal Proliferation.

Grade 0. No evidence of smooth muscle proliferation.

Grade 1. Slight localized smooth muscle growth within the intimal proliferation, comprising no more than 300 to 600 cells per maximum cross section. The cells are large and immature.

Grade 2. Development of smooth muscle cells within the intimal proliferation extending into an area not exceeding one half of the total area of intimal proliferation on cross section.

Grade 3. Growth of smooth muscle cells within the intimal proliferation completed to the extent that it was at times difficult to distinguish normal media from the new structure.

Formation of a New Internal Elastic Membrane.

Grade 0. No trace of a new internal elastic membrane.

Grade 1. Increased density of proliferated elastic lamellas at the lumen.

Grade 2. A definite membrane was distinguishable at the lumen. This was very narrow and did not equal the width of the old internal elastic membrane.

Grade 3. A wide internal elastic membrane at the lumen within newly proliferated intima, almost equal in size to the old internal elastic membrane but staining basically with hematoxylin and eosin.

Grade 4. A wide new-formed internal elastic membrane equal in size to the old internal elastic membrane and showing acidophilic staining with hematoxylin and eosin.

With each rabbit, the grades of change of the six lesions were averaged for each process, and the values are given in the first four columns of table 2. The last two columns, describing regeneration of smooth muscle within the old media and dissolution of medial calcific deposits, indicate merely that these processes were present in most lesions.

The data in table 2 and the data plotted in figure 6 show that intimal proliferation and regeneration of smooth muscle were active fairly early after damage to the aorta. Regeneration of elastic tissue was a slower process, and the formation of a new internal elastic membrane was the

slowest. At the end of six months the regeneration of elastic tissue was still incomplete, although the other processes had nearly reproduced the normal aortic structure. Regeneration of smooth muscle was rapid but

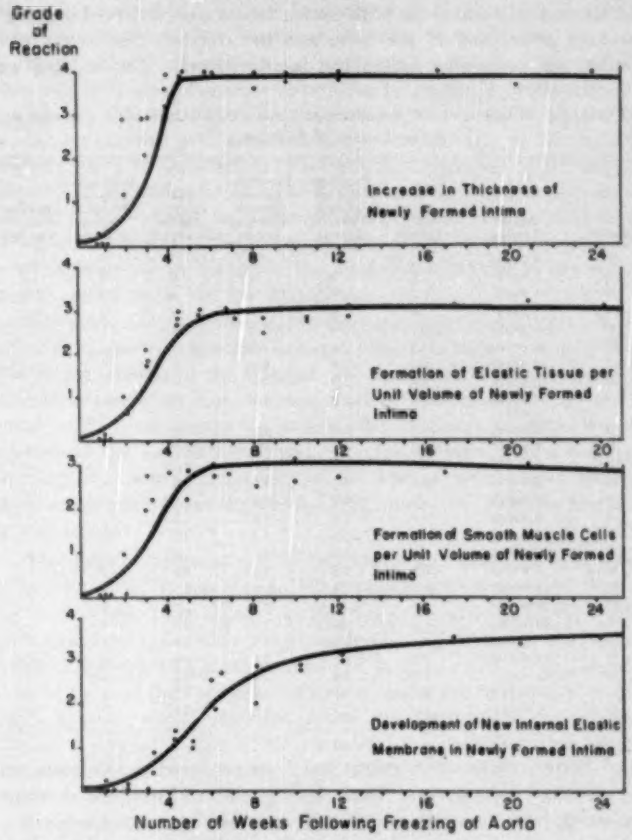
Grade of Reaction
Increase in Thickness of Newly Formed Intima
Formation of Elastic Tissue per Unit Volume of Newly Formed Intima
Formation of Smooth Muscle Cells per Unit Volume of Newly Formed Intima
Development of New Internal Elastic Membrane in Newly Formed Intima
Number of Weeks Following Freezing of Aorta

Fig. 6.—Curves of the magnitudes of the proliferation of the newly formed intima, the formation of elastic tissue and the formation of smooth muscle cells in the newly formed intima. The curves are based on data from table 2.

sparse in the damaged media. Resorption of calcium was evident in most lesions older than four weeks and apparently proceeded slowly.

Although the sequences of histologic changes were not studied in other animals, similar lesions were produced by hypothermal inactiva-

tion of segments of arteries in 2 dogs, 6 months old, and 1 monkey, 6 years old. In these animals, immediately following the production of lesions there was aneurysmal dilatation of the portions of vessels frozen. Intimal proliferation followed by proliferation of smooth muscle cells and elastic tissue within the proliferating intima were observed in a dog's coronary artery and in the aorta and the common iliac artery of a monkey six weeks after lesions had been produced. Another dog was

TABLE 2.—*A Summary of the Average Grades of Regenerative Changes in Aortic Lesions of Increasing Age*

Rabbit	Age of Lesions	Proliferation of Intima	Proliferation of Elastic Tissue in Intima	Proliferation of Smooth Muscle in Intima	New Internal Elastic Membrane	Regeneration of Smooth Muscle in Media	Resorption of Calcium
22	6 days	0	0	0	0	0	..
4	1 week	0	0	0	0	0	..
23	1 week	.3	0	0	0	0	..
30	8 days	0	0	0	0	0	..
32	2 weeks	2.8	1.0	1.0	0	+	..
33	2 weeks	1.2	.4	.8	0	+	..
7	3 weeks	2.4	1.8	2.6	.4	+	..
40	3 weeks	2.0	1.8	2.0	0	+	..
6	3 weeks 1 day	3.0	2.0	2.2	.3	+	0
12	4 weeks	4.0	3.0	2.2	1.2	+	0
29	4 weeks	3.7	3.0	2.3	1.3	+	0
37	4 weeks	3.3	3.0	2.8	1.3	+	+
17	5 weeks	4.0	3.0	2.3	1.0	+	+
9	5 weeks	4.0	3.0	2.8	1.0	+	+
2	6 weeks	4.0	3.0	2.8	2.5	+	0
27	6 weeks	4.0	3.0	2.8	2.3	+	+
41	6⅔ weeks	4.0	3.0	2.8	2.6	+	0
20	8 weeks	3.8	2.5	2.5	2.2	+	+
26	8 weeks	4.0	2.0	3.0	3.0	+	+
25	10 weeks	4.0	3.0	2.7	3.0	+	+
16	10 weeks	4.0	3.0	3.0	3.0	+	+
11	12 weeks	4.0	3.0	3.0	3.3	+	+
15	12 weeks	4.0	3.2	3.0	3.3	+	+
39	17½ weeks	4.0	3.2	3.0	3.5	+	+
35	20 weeks	3.8	3.2	3.0	3.7	+	+
31	24 weeks	4.0	3.3	2.8	3.6	+	+

killed twenty weeks after lesions had been produced in the aorta and the common iliac artery. These lesions showed proliferated intima containing mature smooth muscle cells and thick elastic tissue fibrils.

COMMENT

Human arteriosclerosis is a complex disorder characterized by variable degenerative, regenerative, inflammatory and retrogressive processes in arterial walls. The relations between these processes have not been established, nor is there any reason for believing that the relations and sequences are always the same. The common belief that the

disorder is generalized has led to the concept that it is primarily a systemic disturbance. But a closer scrutiny of the variable distribution of the human disease with accentuation at focal points or in special distributions of branches of the vascular tree leads to a conviction that local factors are perhaps of greater importance than general or systemic factors in determining the origin and evolution of the lesions.

It is generally accepted that arteriosclerotic lesions similar to those often found in human arteriosclerosis can be produced in experimental animals by different methods. As a rule, these methods lead to the production of either a lesion which is most conspicuous in the media of the vessel or a lesion which is principally in the intima. The complete picture of the arteriosclerosis commonly found in the media and intima of aged human vessels has not been reduplicated. This should incite no adverse criticism of the experimental findings, for no disease, bacterial or otherwise ever exhibits the same characteristics in the experimental animal as in the human patient.

Our study of arteriosclerosis in man and experimental animals has led us to believe that the arteriosclerotic lesion is basically a local reaction of components of the vascular wall to influences which upset the balance between functional demand and the functional capacity of the vessel to fulfil the demand. It is implied that this reaction of components of the vascular wall will vary in accordance with the local structure and functional capacity of the wall as well as with local or systemic extramural factors which might modify the ultimate character of the reaction.

The question arose as to how this idea might be tested by a series of experiments. It was decided that rabbits should be used because the work of others has shown that they are perhaps the most susceptible to experimental production of arteriosclerotic and atherosclerotic lesions which closely resemble those encountered in man. Next, it was decided that, in view of the variability of experimental arteriosclerosis of rabbits, a general systemic standard should be established by use of young rabbits, 6 months of age. This eliminated the possibility that senility or spontaneous arteriosclerosis might affect the evolution of the local experimental lesions. Finally, diet and environment were rigidly standardized so that, for practical purposes, most systemic factors which might affect the experiments were controlled. Attention was then directed to production of a local lesion and microscopic study of sequences in the evolution of the lesion. It was decided that the lesions should be produced in the aorta in the general region most susceptible to arteriosclerosis in man, namely, below the level of the origin of the renal arteries. Finally, it was decided that the method of interfering with the structure and the function of the vessel should interfere only

locally with the vascular wall in a reproducible quantitative and qualitative manner without introducing variables such as undue inflammation or anatomic discontinuity of structure. A controlled method of local freezing of the intact normal vascular wall proved to be suitable for these purposes.

After the aortic wall had been activated in discrete areas by freezing, sequences of histologic changes were determined at intervals over a period of six months. These changes were classified as degenerative or regenerative. The degenerative changes were manifested principally as a deterioration of the original structure of the aortic wall. The regenerative changes were manifested principally by a new growth of structure at the site of the lesion. The rate of development and the sequences of these changes were such that there was a continuous change in the nature of the lesion from the beginning to the end of the six month period of observation.

The first effect following freezing of the aortic wall was pronounced aneurysmal dilatation of the frozen segment. This occurred immediately after thawing of the vascular wall. It was attributed primarily to lethal inactivation of smooth muscle of the media with attendant loss of tone. There was no reason for believing that the other components of the aortic wall participated actively in effecting a dilatation. Within a short time, smooth muscle cells began to show acute degenerative changes, so that often by the end of the first or second week the only traces of smooth muscle cells between elastic lamellas in the media were pyknotic fragments of nuclear debris. At no time during this period did thrombi form adjacent to the endothelium of the intima. If the endothelial cells were affected at all, they must have regenerated rapidly, with little histologic evidence of a persistent nature. The collagen and elastic tissue, also, seemed only slightly affected, although collagenous fibrils seemed unduly fused and elastic fibrils unusually straight at the end of the first week. Fusion of collagenous fibrils was most conspicuous in the adventitia, and straightening of the elastic tissue was most pronounced in the media from which smooth muscle cells had disappeared. For some unexplained reason the internal and external elastic membranes were resistant to change and usually remained structurally intact, acting apparently as barriers to ingrowth of fibroblasts and capillaries, *for the usual sequences of inflammatory cell infiltration, vascularization and fibroblastic invasion did not develop in the degenerating, necrotic media.* Instead, such repair as occurred was avascular and characterized principally calcification of zones occupied by nuclear remnants of degenerating smooth muscle cells. In this respect the experimental lesions closely followed the usual pattern of medial degeneration and calcification so often encountered in human senile peripheral arteriosclerosis.

The principal reaction of the aortic wall to injury from the point of view of restoration of function was restricted to the intima. The regenerative reaction here was not accompanied by the usual signs of inflammation. Cells, fibroblastic in form but of unknown origin, actively proliferated here in large numbers. Collagenous and elastic fibrils quickly appeared between these cells. As time passed, the cells assumed the characteristics of smooth muscle cells, and elastic tissue acquired the arrangement and form of elastic lamellas normally found in the media of the aorta. Finally, a new internal elastic membrane developed. A new aortic wall with all its essential components soon formed within the semirigid, partly calcified degenerate shell of the original aortic wall.

Our findings are in accord with the thesis that the arteriosclerotic lesion is a reaction of components of the vascular wall to influences which upset the balance between functional demand and the functional capacity of the vessel to meet the demand. In the present experiments extramural and systemic factors were held reasonably constant. The formation of a new vascular wall occurred promptly without undue interference of extraneous factors or persistent local disturbances.

Every phase of degeneration and regeneration observed in the experimental lesions can be found in a series of human arteriosclerotic lesions. There are, of course, many differences in quality and quantity of reactions between the experimental and the human lesions, but a similarity in the trends or the patterns of degeneration and regeneration cannot be denied. The greatest difference is the absence of atheromatous changes and hyalinized fibrous tissue so often found in human arteriosclerotic lesions. Our studies at the present time are directed toward an investigation of possible causes for these differences. It is possible that the differences are due to limitations of the degree to which the human vascular wall can compensate for the local imbalance of functional demand and functional capacity to fulfil the demand. On the other hand, it is perhaps more likely that the differences are due to interfering extramural or systemic factors that disturb the normal sequence of structural compensatory tissue reactions. In any event, it is now possible to explore the effect of systemic factors such as senility and hypercholesteremia on the local regenerative activity by which a new aortic wall is constructed in the framework of a rapidly proliferating intima of an otherwise almost totally degenerate aortic wall.

SUMMARY

Local segments of the abdominal aorta of the young rabbit were frozen by contact with a cold needle. A microscopic study of the sequence of structural changes occurring in the frozen segments over a

period of twenty-four weeks was made. Continuity of fibrous and elastic structure of the aortic wall was maintained. Inflammation was mild or negligible, and inflammatory cells were restricted to the adventitia. Smooth muscle cells of the media disintegrated, and deposits of calcium appeared in regions occupied by degenerating remnants of these cells. Proliferation of new cells, except for inconstant replacement of smooth muscle in the media, and deposition of new intercellular structure, occurred to a significant degree only in the intima, internal to the old internal elastic membrane. As time passed, a new aortic wall, almost indistinguishable in structural content and thickness from that of the original or undamaged vessel, was regenerated in the framework of the proliferating intima. This appeared to offer almost a perfect functional substitute for the adjacent semirigid, partly calcified degenerate segment of aortic wall.

EXPERIMENTAL VASCULAR DISEASE DUE TO DESOXYCORTICOSTERONE AND ANTERIOR PITUITARY FACTORS

II. Comparison of Pathologic Changes

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THE HYPERTENSIVE vascular disease of human beings has until recent years lacked experimental equivalents in laboratory animals. Arteriopathies have now been shown to occur in the rat following the production of hypertension by silk perinephritis,[1] by injection of desoxycorticosterone [2] and by administration of pituitary preparations.[3] While it has been considered that the hypertension and arterial disease induced with anterior pituitary materials has the same ultimate mechanism as that with desoxycorticosterone, our observations suggest that this is not the case.[4] Consequently, there are available for study three genetically different forms of experimental hypertensive arteriopathy. The present report embodies a comparison of these lesions. In it we seek to demonstrate that their similarities are such that they can be grouped together as representing hypertensive arteriopathy in the rat.

METHODS

Sprague-Dawley albino rats were divided into six groups. Group 1 consisted of male and female rats weighing 80 to 120 Gm. which were subjected to silk wrapping of either one or both kidneys.[5] In some rats the wrapping of one kidney

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1. (*a*) Friedman, B.; Jarman, J., and Klemperer, P.: Am. J. M. Sc. **202**:20, 1941. (*b*) Schroeder, H. A., and Neumann, C.: J. Exper. Med. **75**:513, 1942. (*c*) Cromartie, W. J.: Am. J. M. Sc. **206**:66, 1943.

2. Selye, H.; Hall, C. E., and Rowlev, E. M.: Canad. M. A. J. **49**:88, 1943.

3. Selye, H.: Canad. M. A. J. **50**:426, 1944.

4. Masson, G. M. C.; Corcoran, A. C., and Page, I. H.: J. Lab. & Clin. Med. **34**:1416, 1949.

5. Page, I. H.: J. A. M. A., **113**:2046, 1939.

was accompanied by removal of the other. In most of these animals there ultimately developed a severe type of hypertension, accompanied by vascular lesions. It seemed to us that they would constitute a good reference group for comparative studies. Group 2 consisted of unilaterally nephrectomized female rats treated with desoxycorticosterone acetate (DOCA). This substance, suspended in water, was injected subcutaneously in a daily dose of 2.5 mg. The food consisted of a purchased chow [6a] and the drinking water of 1 per cent sodium chloride solution. Group 3 consisted of female rats unilaterally nephrectomized and then given injections of either a suspension or an alkaline extract of powdered beef anterior pituitary gland.[6b] In order to overcome the effects of antihormones, some animals were given increasing doses of powder or extract: 20 mg. during the first thirteen days, then 40 mg. for ten days and finally 60 mg. until the end of the experiment. Animals of this group were given a diet high in protein (75 per cent chow plus 25 per cent casein) and high in sodium (1 per cent sodium chloride solution as drinking water). As controls for groups 2 and 3, respectively, group 4 consisted of unilaterally nephrectomized rats maintained on a diet high in sodium alone and group 5 on a diet high in protein as well as in sodium.

Blood pressure determinations were made regularly throughout the experiment by the plethysmographic method of Williams, Harrison and Grollman as modified by Kempf and Page [7] or that of Olmsted, Corcoran, Glasser and Page.[8] The blood pressure values of normal rats averaged 99, with extremes of 70 and 130 mm. of mercury.

Animals were killed at various intervals and autopsies made. Tissues were fixed in Zenker's solution and stained with hematoxylin-eosin-methylene blue, Masson's trichrome, phosphotungstic acid-hematoxylin, Weigert's fibrin and elastic stain.

GROUP 1. RESULTS OF KIDNEY WRAPPING

Of 85 rats in this group, 55 survived long enough for hypertension to develop. Death from renal insufficiency was particularly common in animals with one kidney removed and the other wrapped. Various degrees of renal atrophy were observed, but this was most evident in those animals in which one kidney was wrapped and the other intact, and the average weight of the wrapped kidney was 611 mg. (520-1,180 mg.) while that of the intact one was 1,228 mg. (1,020-1,570 mg.). Hypertension appeared as soon in these as in animals unilaterally nephrectomized or with both kidneys wrapped. It reached levels of 140 to 150 mg. of mercury at twenty to twenty-six days. The degree of atrophy did not seem to parallel the increase in blood pressure; e. g., one rat with a difference of 300 mg. between the two kidneys had a blood pressure of 175 mm. of mercury while another examined at the same postoperative period had a blood pressure of 148 mm. with a

6. (*a*) The chow was the fox chow marketed by the Ralston Purina Company, St. Louis, Mo., and Woodstock, Ont., Canada. (*b*) The beef anterior pituitary powder was purchased from Desbergers Limited, Montreal, Canada.

7. Kempf, G. F., and Page, I. H.: J. Lab. & Clin. Med. **27**:1192, 1942.

8. Olmsted, R.; Corcoran, A. C.; Glasser, O., and Page, I. H.: Federation Proc. **7**:88, 1948.

difference of 600 mg. in kidney weights. The atrophic kidneys were abscess free and grossly similar.

When hypertension became severe, many of the rats had hematuria, lost appetite and weight, became cachectic and died.

Macroscopic Examination.—The wrapped kidney, although variably atrophic and with a slightly granular surface, seemed relatively normal. On the other hand, the intact kidney was grossly abnormal; it was large and of a grayish color, and its surface was irregular and mottled with small white or hemorrhagic points.

The heart was frequently enlarged, especially when hypertension was of long standing. In the cases of most severe hypertension its surface was irregular and deformed by white patches; these were mainly localized to the right side near the apex. The aorta was somewhat enlarged, elongated and inelastic. In 1 rat with both kidneys wrapped, early and severe hypertension was observed (160 mm. of mercury on the fifteenth day; 216 mm. on the fifty-first day), and its hard, deformed aorta histologically revealed widespread medial calcification.

Splanchnic arteries, particularly the hepatic, duodenal, pancreatic, splenic and superior mesenteric arteries, were especially prone to lesions. An early detectable macroscopic change consisted in the appearance of a slight gelatinous and opalescent halo around the mesenteric vessels. In the next stage, small white nodes appeared at the bifurcations of the mesenteric or at the origins of the hepatic or duodenal arteries and at various sites in the vessels and at different distances from the aorta. Later these lesions became confluent with hemorrhagic areas. In the last stage the arteries appeared as tortuous, deformed cords with diameters measuring up to 5 mm.

The lesions were present in 83 per cent of the rats with one kidney wrapped and the other removed, 85 per cent of those with both kidneys wrapped and only 18 per cent of those with only one kidney wrapped and the other untouched. This distribution is in accord with the remote effects of renal infarction, in which the incidence of extrarenal arterial disease is inversely proportional to residual normal kidney mass.[9] As concerns duration, lesions were present in all rats with chronic hypertension (more than 150 mm. of mercury) of more than three months' duration, regardless of its severity. While the observations summarized in table 1 must be supplemented before a definite conclusion can be reached, it seems likely that there are other important pathogenetic factors besides duration and severity of hypertension, such as individual sensitivity or suddenness of the rise in blood pressure.

The other organs did not show macroscopic lesions, except the brain and the gut, which sometimes presented hemorrhages.

9. Loomis, D.: Arch. Path. 41:231, 1946.

Microscopic Examination.—1. Kidney: The changes varied greatly, depending on the tension of the silk wrapping and the duration of the experiment. The less severe lesions consisted only of a moderate focal dilatation of tubules associated with albuminous casts, and the more severe, of necrosis, hyalinization and sclerosis of glomeruli, hydropic degeneration and atrophy of tubules associated with a disproportionate tubular dilatation and accumulation of hyaline casts.

The renal lesions were always most severe at the poles, where, no doubt, the compression of the parenchyma by the silk was greater than over the convexity. Particularly at the poles, fibrous tissue invaded the cortex; within it lay remnants of nephrons. The tubular epithelium in these was grossly normal but had lost eosinophilic granulations and brush border. The reaction for alkaline phosphatase was negative in these areas, which suggests that these remnants had no excretory function.

Formation of a thick fibrocollagenous hull, so evident in dogs with silk perinephritis, was minimal in the rat.

The following protocols of individual rats exemplify the variety and range of renal lesions encountered. The first two portray two forms of lesions observed early in the disease, and the last, lesions in chronic hypertension.

Rat 270: Both kidneys were wrapped. The blood pressure rose to 160 mm. of mercury on the fifteenth day and increased slowly to exceed 200 mm. at the end of the experiment (fifty-first day). At autopsy there were generalized lesions of arteritis in the viscera.

The kidneys showed superficial and localized areas of inflammation, fibrosis and calcification. Only remnants of tubules and few glomeruli remained in the scarred areas at the poles. In the rest of the cortex there was generalized and severe dilatation of tubules; most of these were empty except for a few which contained granular or homogeneous pink-staining material. The tubular cells were reduced in size, many were atrophic, and some showed early albuminous droplet degeneration. The glomeruli were enlarged, but the tufts were atrophic, so that the capsular space was increased in size. This space was either empty or filled with eosinophilic material. Some of the tufts showed focal necrosis.

Rat 234: One kidney was wrapped; the other, removed. The blood pressure reached 208 mm. of mercury on the fifty-second day and remained at this level until the sixty-sixth day, when the animal was killed. This rat had hematuria; at autopsy there were arteritic lesions and hemorrhages in the brain, the intestinal tract, the adrenal glands and lymph nodes.

The renal lesions were of an acute type. As in the preceding rat, there was polar fibrosis. Glomeruli were greatly enlarged either by accumulation of blood in the subcapsular space or by swelling of the tufts. Many of these glomeruli presented lesions of necrotizing glomerulitis; in some the tuft was constituted of large foamy cells and was adherent to the capsule, which showed focal proliferation. Many tubular cells showed atrophy or hydropic degeneration; on the other hand, numerous mitotic figures indicated that some cells of proximal convoluted

tubules were regenerating. The latter cells also were losing their brush border and eosinophilic granules and seemed to be forming papilloma-like structures similar to those described by Selye for the "endocrine kidney."[10]

In the interstitial tissue there was focal collection of lymphocytes and polymorphonuclear cells. In spite of periarteritis and panarteritis of the renal artery, the intrarenal blood vessels were only slightly affected; a few arterioles showed complete homogenization of their walls.

Rat 18: Both kidneys were wrapped. The blood pressure reached levels of 160 mm. of mercury during the third month and gradually rose to 200 mm. until the animal was killed on the one hundred and thirty-ninth day.

Changes in the polar zones were identical with those previously described. In the rest of the kidney the glomeruli were enlarged and showed signs of necrosis and hyalinization. In some of them there were prominence of the capsular epithelium and thickening of the basement membrane, which was adherent to the tuft so that the capsular space was completely obliterated. Here the tuft was composed of large globular cells containing red granules and of amorphous masses of fibrinoid material; only one or two patent capillaries were visible. In some glomeruli there were sclerotic changes so advanced that there was complete loss of structure.

The blood vessels showed all stages of arterionecrosis and arteriolonecrosis. The initial lesion seemed to be an infiltration of protein-rich fluid resembling plasma between the endothelium and the elastic membrane, accompanied by an increase in the diameter of the blood vessel. The infiltrative and necrotic process extended to the rest of the wall with destruction of the media, followed by complete repair by formation of fibrous tissue. In a longitudinal section, beadlike formations constituted of fibrous tissue appeared along an intralobular artery (fig. 1). Arcuate and intralobar arteries presented only slight medial hypertrophy.

2. Heart: In brief experiments, cardiac lesions were not frequent as were lesions of the kidneys or of the mesenteric and pancreatic arteries. However, in chronic hypertension there was often necrosis of the myocardium or hemorrhages which ultimately resulted in areas of scarring that were detectable on gross examination. Lesions of the blood vessels, most common in the right ventricle, consisted of fibrinoid degeneration or necrosis of small vessels, with ovoid areas of perivascular proliferation and exudation, measuring up to 600 microns in length, sometimes enclosing the damaged vessel (fig. 5). The vessels affected were 30 to 60 microns in total diameter; their walls were markedly, mostly concentrically, thickened, owing to deposition of fibrinoid material, contained a few polymorphonuclear neutrophilic cells of the granulocytic series and did not show necrosis. The lumens were much diminished in size or obliterated, the endothelial cells frequently surviving as units crowded in the reduced lumen. There was a compact perivascular cuff of proliferating fibroblastic tissue up to 70 microns in width, containing lymphocytes, a few polymorphonuclear neutrophilic cells and, marginally, degenerating cardiac muscle fibers and Anitschkoff cells. At times the

10. Selye, H., in Conference on Factors Regulating Blood Pressure, Transactions of the Second Conference, Jan. 8-9, 1948, New York, Josiah Macy, Jr., Foundation, 1948.

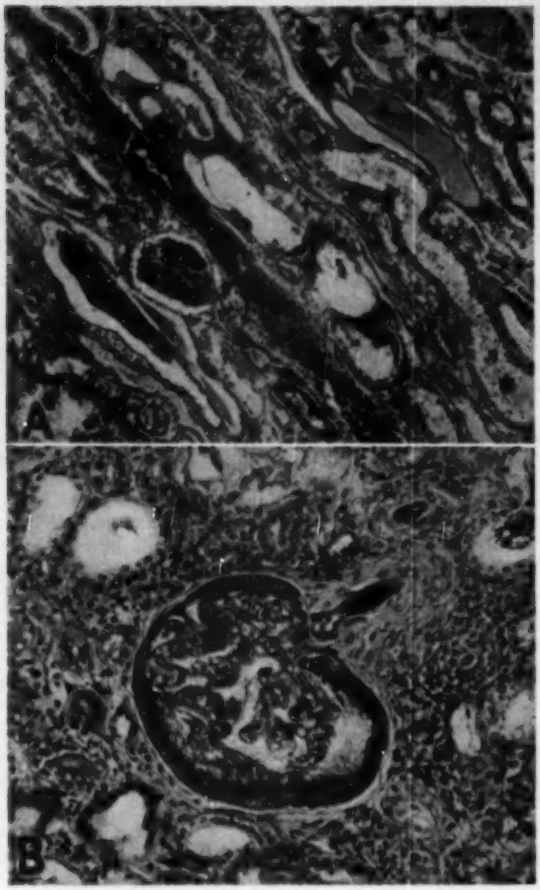

Fig. 1.—*A*, kidney from a uninephrectomized rat with one perinephritic kidney. Various stages of necrotizing arteriolitis are shown on a longitudinal section of an interlobular artery. There are focal necrosis, deposition of fibrinoid and focal proliferative reaction in the vessel wall. Tubules are dilated and contain hyaline and cellular casts; the epithelium shows atrophy and hydropic degeneration. The glomerulus is relatively normal. Trichrome stain; × 70.

B, kidney from a rat treated with an anterior pituitary preparation, showing a juxtaglomerular arteriole with intimal fibrinoid deposits and marked fibroblastic thickening. The glomerular tuft is partly edematous and necrotic, with collapsed capillaries; the capsular space is filled with abundant fibrinoid deposit. There is mild chronic interstitial nephritis. Hematoxylin and eosin; × 150.

adventitial zone was formed by fibrocytes and moderately dense collagen, with an outer thin cell and exudate layer. In some instances there was a focus of fibrinoid material adjoined by necrotic cardiac muscle with hemorrhage and a dense infiltrate of polymorphonuclear neutrophils.

Rarely a small vessel was found with fibrinoid thickening or necrosis of the wall, but without appreciable perivascular proliferation or exudate.

Occurring principally in the left ventricular wall and interventricular septum, there were elongate foci, measuring up to approximately 250 microns, comprised of cellular edematous or somewhat loosely arranged connective tissue with a slight lymphocytic exudate, and containing rare, degenerating muscle fibers. Occasionally, small vessels of a total diameter of about 25 microns were enclosed in these and showed edema

TABLE 1.—*Effects of Blood Pressure Levels on the Incidence and the Severity of Arteritis in the Mesentery and the Pancreas*

Rat	Blood Pressure Levels During the Indicated Postoperative Periods						Severity of Arteritis, 0 to ++
	10-20 Days	20-30 Days	30-40 Days	40-50 Days	50-80 Days	80-100 Days	
259	154	160	158	150	148	190	++
265	202	174	184	190	190	...	0
266	170	190	178	200	184	192	+
267	176	186	170	176	210	...	++
270	160	154	154	160	216	...	++
273	140	166	172	210	168	178	0
279	142	160	170	170	182	210	+
290	186	176	180	188	192	...	+
292	180	214	210	...	...	...	0
299	180	220	216	216	...	...	0

with slight lamination of the walls or fibrinoid change. Infrequently there was a vessel approximately 50 microns in caliber that showed marked hydropic change in the subendothelial zone, reduction in the size of the lumen and a very slight perivascular lymphocytic exudate. At times these vessels revealed, in addition, small eccentric foci of fibrinoid change involving the media and the subendothelial area.

The stages of the evolution of these vascular changes are difficult to evaluate: Neither the incidence nor the character of the lesions is related to the degree or the duration of the hypertension or to lesions elsewhere in the vascular tree. In this respect rats 6 and 18 are good examples. Both had panarteritis and polyarteritis of the mesenteric arteries, but rat 18 with hypertension of one hundred and fifty-nine days' duration presented slight necrosis of coronary vessels, while rat 6 with hypertension of thirty days' duration had extensive perivascular granulomatous proliferations with severe necrosis of myocardial vessels and cardiac parenchyma.

3. Blood Vessels: The invasiveness of the mesenteric and pancreatic lesions permitted a full view of the various stages of their evolution in a single histologic section. The stages seemed to be as follows: (1) perivascular collection of polymorphonuclear cells accompanying hypertrophy or hydrops of endothelial and medial cells and occasional minute medial hemorrhages; (2) proliferation of perivascular tissue; (3) necrosis of the media with polymorphonuclear cell invasion; (4) fulminant inflammatory reaction involving the whole wall and the perivascular tissue; this was associated with appearance of an inflammatory exudate rich in pleomorphic cells; (5) subintimal accumulation of fibrinoid material; (6) localized or generalized intimal fibroblastic proliferation, which sometimes obliterated the lumen almost completely and resulted in formation of thrombi; (7) healing by formation of scar tissue and ultimately calcification.

Arteries and arterioles were first involved, but inflammatory reactions were also seen around veins and pancreatic and bile ducts as a result of their contiguity to vessels. Arteriolonecrosis in the intestinal wall is a possible sequence of the mesenteric polyarteritis and the immediate cause of intestinal hemorrhages.

The morphologic aspect of the adrenal glands is under detailed study by Dr. Helen Deane. Since these observations will, in any case, bear more on pathogenesis than on the arterial disease, they will form the subject of another report.

GROUP 2: RESULTS OF DESOXYCORTICOSTERONE TREATMENT OF RATS GROUP 4: CONTROL RATS

The lesions in this experimental group differed significantly from those in the rats with renal hypertension and those in the rats with pituitary hypertension in their greater severity and in their regularity even in experiments of short duration. This method of producing hypertension and vascular lesions has advantages over those involving surgical treatment of the kidney or the renal artery, chiefly in the fact that the causative factors (desoxycorticosterone, high sodium diet) are readily quantitated.

Unilaterally nephrectomized female rats receiving 1 per cent chloride solution were treated with 2.5 mg. of desoxycorticosterone acetate daily. Blood pressure was determined regularly, and animals were killed at various intervals, starting on the eighth day, in an attempt to detect the first morphologic evidence of a lesion.

Polydypsia and polyuria appeared within a few days after the treatment was begun and persisted throughout. Around the fifteenth day tics developed in some animals and then gradually disappeared. Whenever they reappeared, they were more severe; animals had difficulty in eating, became rapidly emaciated and had to be put to death. In experi-

ments of long duration some rats became comatose and were relieved when given tap water to drink for a few days instead of sodium chloride solution. Possibly because of an air-conditioned environment, we never observed the joint swelling described by Selye, Sylvester, Hall and Leblond.[11]

At autopsy the animals treated for a period of eight to twenty days did not show any gross lesions except an increase in size of the kidney, which otherwise had a normal appearance. When treatment was prolonged, the kidney, the heart and gastrointestinal arteries showed progressive signs of damage: the surface of the kidney became granular, and its color turned from red brown to pale gray; the heart and arteries presented the same gross changes as in rats with kidneys

TABLE 2.—*Effects of Duration of Desoxycorticosterone Treatment on Blood Pressure and Organ Weight*

Duration, Days	Last Blood Pressure, Mm. Hg.	Kidney Wt. per 100 Sq. Cm.	Heart Wt. per 100 Gm.	Adrenal Wt. per 100 Gm.
0	110 (80-120)	320 (260-380)	350 (300-400)	31 (17-35)
8	140 (120-160)	330 (290-360)	370 (340-390)	24 (20-25)
14	135 (128-160)	320 (290-340)	320 (300-350)	21 (17-24)
20	135 (140-155)	440 (400-460)	450 (380-480)	19 (16-20)
27	156 (138-170)	360 (340-380)	430 (400-450)	16 (14-20)
53	199 (186-212)	556 (466-662)	413 (365-473)	28 (19-36)

wrapped. Furthermore, the animals became edematous and showed ascites and often hydrothorax and hydropericardium as well.

In table 2 we give the average blood pressure and the average organ weights of animals treated with desoxycorticosterone for various lengths of time. The blood pressure and the heart and kidney weights increased as treatment was prolonged, while the adrenal glands decreased in size. The relative increase found on the fifty-third day may be attributed to the cachexia and loss of weight resulting from the toxic effect of desoxycorticosterone or from the hypertensive state or from both.

Microscopic Examination.—The kidneys of the control, untreated group (group 4) receiving 1 per cent sodium chloride solution revealed a slight focal dilatation of convoluted tubules. These tubules were filled with cell debris consisting of amorphous chromophobic material in which nuclei were sometimes present. The cell changes in the dilated part of the tubules consisted of a swelling of the cytoplasm bordering the lumen,

11. Selye, H.; Sylvester, O.; Hall, C. E., and Leblond, C. L.: J. A. M. A. **124**:201, 1944.

into which it protruded, and a loss of eosinophilic granules in the apical part of the cell. These hydropic cells, even though capable of mitotic fission, were often seen to be detached from the basement membrane. Cells which did not undergo these changes would remain apparently normal; however, their nuclei would shrink and stain dark in contrast to the nuclei of the hydropic cells, which were large, vacuolated and lightly stained. These morphologic alterations were most evident during the first fifteen days, at the time when compensatory renal hypertrophy was more rapid. The glomeruli showed only a slight increase of their cellularity.

The heart and the pancreas were normal.

Histologic examination of organs from desoxycorticosterone-treated rats killed before the fifty-third day of the experiment, when the blood pressure had already increased, did not reveal renal changes significantly different from those seen in controls. The hypertension, therefore, may not be attributed to renal vascular lesions. Rather, these lesions would seem to result from, or at least to follow in the wake of, arterial hypertension. The most striking change in control and experimental groups alike was dilatation of tubules, which at first was slight, focal and restricted to the convoluted tubules, then became more extensive, with a tendency to spread to collecting tubules. The cellular changes in the epithelium of the convoluted tubules were at first the same in all groups. Later in experimental groups the epithelium became atrophic with loss of luminal eosinophilic granulations; the nuclei shrank, became few and bulged through the atrophic cytoplasm. This atrophy was not necessarily associated with local accumulations of casts. The round, dark nucleus of many cells was surrounded by a halo, so that they corresponded to those described by Knowlton, Stoerk, Seegal and Loeb.[12] In places the periglomerular convoluted tubules became basophilic; in these areas the interstitial tissue was infiltrated by round cells and fibroblasts, the tubular cells lost their brush borders and eosinophilic granules, and the tubular lumens were almost completely occluded by proliferating epithelium. The casts found during the first fifteen days were composed of cell debris; then few hyaline or blood casts were observed. Glomeruli showed an increase in cellularity, and many were hypertrophied and presented mitotic figures. The capsular spaces of some of them contained small amounts of granular material or blood. In few rats we noticed chronic focal interstitial nephritis and fibroblastic hyperplasia localized to the cortex. Whenever arterioles were visible in these areas, they did not show any of the alterations characteristic of hypertensive cardiovascular diseases.

12. Knowlton, A. I.; Stoerk, H.; Seegal, B. C., and Loeb, E. N.: Endocrinology **38**:315, 1946.

The pancreas and the mesentery showed perivascular proliferation and signs of chronic inflammation as early as eight days after the beginning of treatment. Since these changes were seen in some control rats, they were perhaps the result of the surgical intervention performed at the beginning of the experiment. We did not notice any lesions of acute arteritis in the abdominal arteries.

The first lesion of the heart was found on the twentieth day of the experiment and consisted of necrotizing arteriolitis with perivascular proliferation and parenchymal lesions with necrosis and fibroblastic proliferation. The last three blood pressure values of such a rat were 145, 165 and 155 mm. of mercury. Another rat with the same cardiac changes was found on the twenty-seventh day; its blood pressure varied from 150 to 160 mm.

On the fifty-third day of the experiment, all the desoxycorticosterone-treated rats showed the functional and morphologic characteristics of hypertensive cardiovascular disease (fig. 2). Renal tubules were greatly dilated and filled with blood or with homogeneous hyaline casts which stained blue with hematoxylin-eosin–methylene blue. Most of the glomeruli were large and anemic, and filled Bowman's capsule. The capsular space was either obliterated or greatly distended by accumulation of blood or hyaline material, and the capsular membrane was thickened. The tufts were either necrotic or partially or completely hyalinized; while cells decreased in number they increased in size due to a swelling of their cytoplasm. This was accompanied by obliteration of most of the capillaries. In some areas of the cortex, lumens of proximal convoluted tubules were filled with proliferating epithelial cells. Between the degenerated convoluted tubules the arteries and arterioles were very prominent and diseased. The lesions were those of necrotizing arteritis and arteriolitis, with no inflammatory cells infiltrating the blood vessel wall. Among the renal arteries, only the intralobular arteries were affected by this necrotizing process; arcuate and intralobar arteries presented minimal changes in the form of medial hypertrophy. The juxtaglomerular apparatus, which in short term experiments was often hypertrophic, was either necrotic or fibrotic at the end of the experiment. We frequently observed edema of the pelvis and occasionally pyelonephritis.

In the heart there were focal necrosis of the myocardium and fibroblastic proliferation at the site of old hemorrhages, evidenced by the presence of blood pigment. Individual muscle fibers were hypertrophied. There was necrotizing arteriolitis and extensive, invasive perivascular proliferation (fig. 5). In the arteries of the pancreas and the mesentery there were acute lesions of necrotizing arteritis indistinguishable from those seen after kidney wrapping (fig. 4). In 3 rats vascular changes were present in all the organs that we examined: heart, kidney,

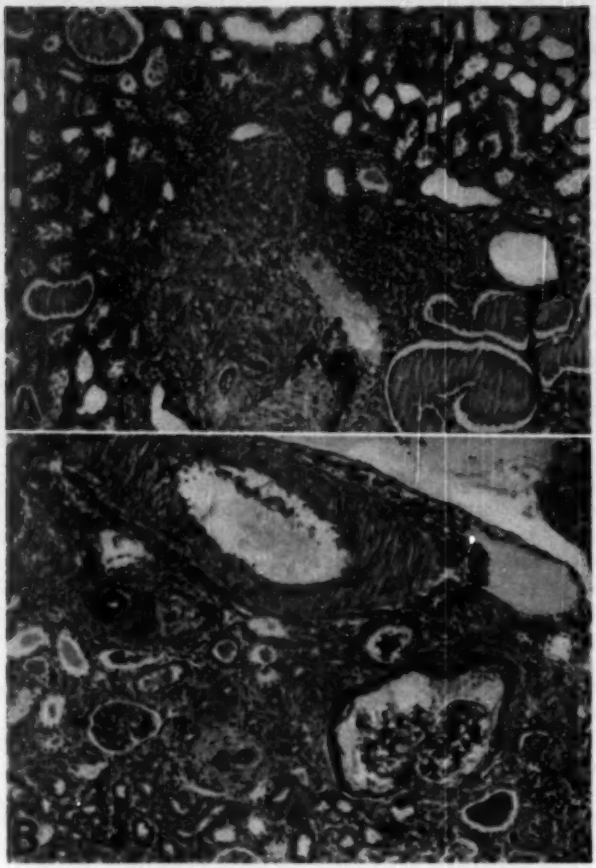

Fig. 2.—*A*, kidney from a rat treated with desoxycorticosterone, showing necrotizing arteriolitis, glomerulitis, dilatation of tubules, hyaline casts and interstitial nephritis. The glomerulus completely fills the capsular space and is adherent to the capsule. The arteriole is tortuous and markedly thickened because of fibrosis and deposition of fibrinoid. Hematoxylin and eosin; × 70.

B, kidney from a rat treated with an anterior pituitary preparation, showing various stages of necrotizing arteriolitis. The arteriolar wall is thickened as a result of either fibrinoid necrosis or connective tissue proliferation or both. The glomerulus is collapsed and shows fibrinoid deposit on the capsule and in the intercapillary zone of the tuft. Tubules are moderately dilated and contain hyaline casts. There is slight chronic interstitial nephritis. An arcuate artery shows only medial hypertrophy. Trichrome stain; × 70.

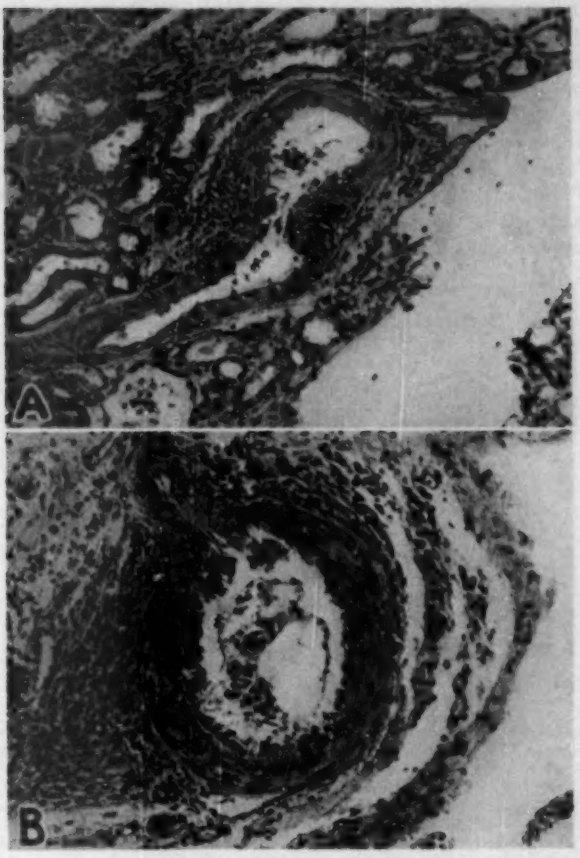

Fig. 3.—Sections of kidney of a rat with silk perinephritis: *A*, early arterial necrosis at the junction of an arcuate and an interlobular artery. The wall is infiltrated with polymorphonuclear neutrophilic cells and is edematous; the endothelial cells are swollen and prominent. Hematoxylin and eosin; × 125.

B, junction of a branch of the renal artery and an interlobar artery. Note focal early necrosis of the arterial wall accompanied by mobilization of polymorphonuclear neutrophilic cells and intimal deposition of fibrinoid. Note also thickening of the adventitia due to inflammatory cells and fibroblastic proliferation. Hematoxylin and eosin; × 125.

uterus, skin, periarticular tissue and brain. In the last organ there resulted encephalomalacia and cyst formation. In preparations stained with phosphotungstic acid–hematoxylin one could observe a fibrinoid exudate extending widely around the arterioles and capillaries, with necrosis of the wall and obliteration of the lumen (fig. 6). Similar lesions of the brain have been described by Scheinker, who considered them a specific form of hypertensive arteriolopathy.[13]

These pathologic changes which appeared after fifty-three days of daily treatment with 2.5 mg. of desoxycorticosterone acetate could be demonstrated in twenty-nine days when the dose of the acetate was increased to 5 mg. per day. In 2 rats we studied the effect of cessation

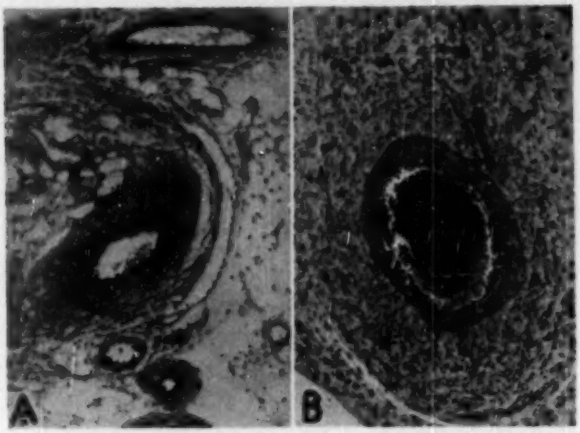

Fig. 4.—*A*, mesentery from a rat treated with desoxycorticosterone, showing an early stage of necrotizing vascular lesions in artery and arteriole. There are perivascular proliferation and medial hypertrophy. The elastic membrane is still intact. Weigert elastic stain and trichrome stain; × 70.

B, mesentery from a rat treated with an anterior pituitary preparation, showing an acute stage of necrotizing panarteritis. There is necrosis of the arterial wall with accumulation of inflammatory cells and subintimal deposition of fibrinoid. Trichrome stain; × 70.

of treatment on the evolution of hypertension and vascular lesions. The blood pressure values at the end of twenty-nine days averaged 186 mm. of mercury in rat 7 and 188 mm. in rat 8. Biopsy of a mesenteric artery of each rat revealed acute arteritic lesions. Macroscopically, the kidneys appeared sclerotic. Sodium chloride solution was replaced by tap water at the time injections of desoxycorticosterone acetate were discontinued.

13. Scheinker, I. M.: Ann. Int. Med. **28**:630, 1948.

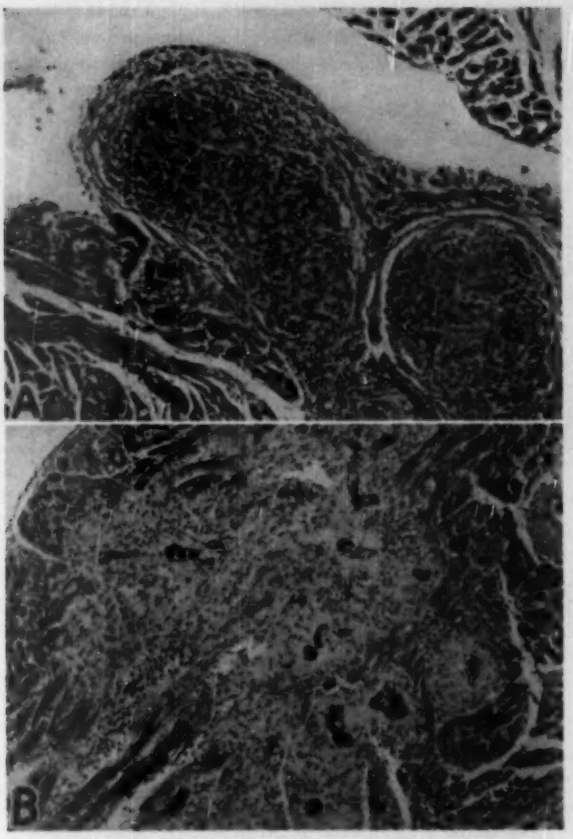

Fig. 5.—*A*, right ventricular myocardium of a rat treated with desoxycorticosterone, showing tortuosity and impressive thickening of arteriolar walls due to cellular infiltration and fibroblastic proliferation. The lumens are obliterated, and the perivascular zone is edematous and infiltrated by fibroblasts and inflammatory cells, mostly lymphocytes. Hematoxylin and eosin; × 100.

B, right ventricular myocardium of a rat with kidney wrapping, showing extensive loss of muscle fibers. In most of the arterioles fibrinoid is being deposited in the intima, and there is fibroblastic proliferation in the wall and the perivascular area. In the upper center there is a larger vessel showing only hypertrophy of its wall and a slight adventitial proliferation. Phosphotungstic acid–hematoxylin; × 70.

In both animals the blood pressure remained stationary for ten days and then fell slowly to levels of 140 mm. of mercury on the fifty-second day, when the rats were killed. On histologic examination, rat 7 had malignant nephrosclerosis, necrotizing periarteritis and panarteritis, while rat

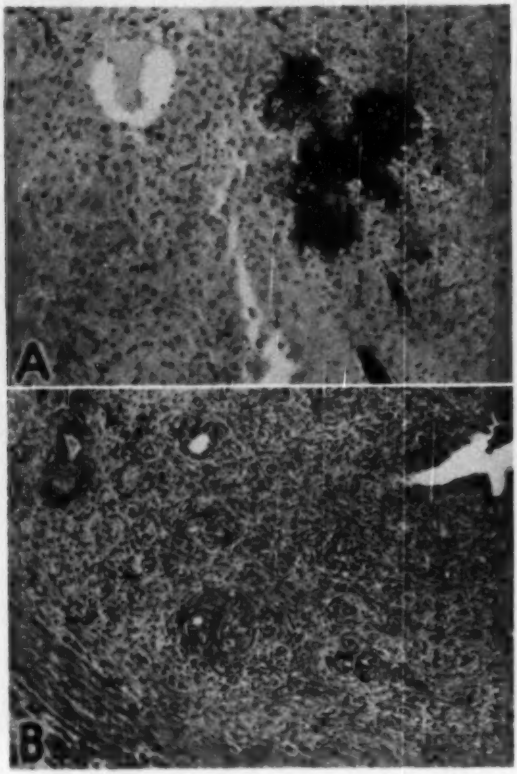

Fig. 6.—*A*, brain of a rat treated with desoxycorticosterone, showing an area of encephalomalacia. Note the extensive fibrinoid exudate obliterating capillaries, and a small cyst. Phosphotungstic acid–hematoxylin; × 70.

B, uterus of a rat treated with desoxycorticosterone, showing tortuous arterioles with walls thickened by deposits of fibrinoid. There is a moderate cellular infiltrate in the adjoining connective tissue. Hematoxylin and eosin; × 70.

8 had focal sclerosis, interstitial nephritis, great dilatation of tubules, chronic arteritis and periarteritis. Therefore, discontinuance of the

administration of desoxycorticosterone acetate and sodium chloride brought a decrease in blood pressure without definitive regression of the vascular lesions.

In another series of experiments we studied the effect of desoxycorticosterone given to 7 rats with one kidney wrapped and the other intact. The daily dose of the acetate was 2.5 mg. per day; furthermore, the animals were given 1 per cent sodium chloride solution to drink. A control group of 8 rats with one kidney wrapped but not receiving the steroid was observed simultaneously. Autopsies were performed on the thirtieth day. The results are summarized in table 3.

It can be seen that a dose of desoxycorticosterone acetate which by itself was insufficient to cause vascular lesions in thirty days was able to increase the incidence and the severity of lesions in rats with one

TABLE 3.—*Effects of Desoxycorticosterone Administered to Rats in Which Unilateral Perinephritis Had Been Produced*

Treatment	Blood Pressure, Mm. Hg.		Organ Weights, Mg.			Incidence of Arteritis, per Cent
	25th Day	30th Day	Left * Kidney	Right Kidney	Heart	
None	141 (130-167)	146 (128-175)	613 (320-1,180)	1,208 (1,000-1,570)	777 (690-940)	18
Desoxycorticosterone	147 (133-178)	174 (156-187)	820 (710-950)	1,380 (980-1,600)	813 (600-1,155)	100

* This was the wrapped kidney.

kidney wrapped. Even though blood pressure was not extremely high in rats receiving the steroid and lesions in both kidneys were mild, the vascular and cardiac lesions were severe.

GROUP 3: RESULTS OF ANTERIOR PITUITARY TREATMENT
GROUP 5: CONTROL RATS

Unilaterally nephrectomized rats were given injections of a crude suspension or an alkaline extract of powdered anterior pituitary gland. The daily dose level was either kept constant or raised as the experiment progressed. Since the lesions observed under these various conditions did not differ significantly, we shall describe their evolution chronologically.

Untreated control animals with a high protein, high sodium intake (group 5) which were killed at the same postoperative intervals as the experimental rats did not show pathologic changes different from those seen in animals of group 4. In the kidney there were increased cellularity in the glomerular tufts, tubular dilatation and occasional focal interstitial nephritis. In 1 of 30 rats we found a few hyalinized glomeruli and partial obliteration of the capsular space owing to adhesions between the tuft and the capsule. Its blood pressure varied from 84 to 122 mm.

of mercury. The lesions may have been spontaneous and not results of the high sodium, high protein diet.[14] No pathologic alterations were observed in any other organ.

Under the influence of pituitary treatment, gross renal changes appeared around the twenty-fifth day. Kidneys were enlarged, edematous, pale gray, with an irregular, mottled surface. As treatment progressed, these changes became more prominent, and white or red points were visible. The heart was enlarged and in some instances presented white streaks on the right side. Arterial thickening due to arteritis was seen in the pancreas in only 4 rats of 66 (fig. 4). It did not occur in the mesentery. Two of these rats had duodenal hemorrhages. The adrenal glands were abnormally large, weighing more than 150 mg. in animals with blood pressures over 150 mm. of mercury. They were partly hemorrhagic or necrotic, probably because of vascular lesions. The pituitary gland was small as a result of compensatory atrophy.

On histologic examination the first detectable change was seen in the kidneys of rats treated for a period of ten days. They were identical with those observed in controls and consisted of tubular dilatation and focal lymphocytic infiltration. Blood pressure was slightly elevated over the preoperative levels, reaching values of 120 mm. of mercury.

In the twenty day experiment the preceding changes were more accentuated; there were also increases in the cellularity of glomeruli, and granular casts.

When treatment was prolonged, animals responded irregularly. In the majority of them the lesions were stationary; such animals had a normal or slightly elevated blood pressure. In others the renal lesions became more severe. Casts were more homogeneous and stained blue with methylene blue. Glomeruli enlarged, and necrotizing glomerulitis took place, at first focal and then generalized. In some animals there were glomerular and tubular hemorrhages. Capsular spaces were filled with granular material, which became more homogeneous. Owing to the proliferation and swelling of the tufts, fusion of the glomerulus and the capsule, partial or complete, gradually took place. Then there was progressive hyalinization of the tufts with plugging of the capillary loops and thickening of the glomerular membrane. At this stage the lesions could not be distinguished from those obtained with desoxycorticosterone (figs. 1 and 2).

Hyalinization of the glomeruli was accompanied with lesions of the afferent and intralobular arteries. Arcuate and larger arteries were relatively unaltered. These lesions were those of necrotizing arteritis and fibrosis, involving the juxtaglomerular apparatus. Inflammatory

14. Griffith, J., and Farris, E. J.: The Rat, Philadelphia, J. B. Lippincott Company, 1942.

cells did not participate in the evolution of these lesions, although they were frequently seen in interstitial tissues. Pyelonephritis occurred in a few cases. Again, there is a great similarity between the pathologic changes of these kidneys and those seen in desoxycorticosterone-treated rats.

COMMENT

Malignant nephrosclerosis of human beings is characterized by the following lesions: (1) necrosis and hyalinization of the glomeruli; (2) fibrinoid necrosis of afferent arterioles and intralobular arteries without perivascular inflammatory cell infiltration, and (3) only slight changes (elastosis) in arcuate and larger arteries (Schürmann and MacMahon [15]; MacMahon and Pratt [16]). When these criteria are applied to the renal tissues obtained from rats after kidney wrapping or injections of desoxycorticosterone acetate or injections of anterior pituitary materials, the experimental lesions can be construed only as identical with those of the clinical disease. Such lesions have already been described as occurring in rats subsequent to kidney wrapping (Friedman, Jarman and Klemperer [1a]; Schroeder and Neumann [1b]; Cromartie [1c]), renal infarction (Loomis [9]), partial constriction of the renal artery (Wilson and Byrom [17]; Schroeder and Neumann [1b]; Schloss [18]) or choline deficiency (Hartroft and Best [19]).

Selye, Hall and Rowley [2] were the first to describe the nephrosclerosis produced in rats by administration of desoxycorticosterone. Their demonstration has been confirmed in detail by our experiments, which, it will be noted, were done under identical conditions. Failure to elicit nephrosclerosis with desoxycorticosterone has been reported by Knowlton, Stoerk, Seegal and Loeb [12] and Bechgaard and Bergstrand.[20] However, a survey of these reports establishes the fact that the doses of desoxycorticosterone or sodium chloride solution or both were much less than those used by Selye or by us and that in some series the rats were not unilaterally nephrectomized.

Selye and co-workers [21] also reported that crude anterior pituitary preparations were either renotropic or nephrosclerotic, depending on the protein content of the diet. The latter effect appeared when rats were unilaterally nephrectomized and fed a high protein, high sodium diet. However, alkaline pituitary extracts were less effective than a

15. Schürmann, P., and MacMahon, H. E.: Virchows Arch. f. path. Anat. **291**:47, 1933.

16. MacMahon, H. E., and Pratt, J. H.: Am. J. M. Sc. **189**:221, 1935.

17. Wilson, C., and Byrom, F. B.: Quart. J. Med. **10**:65, 1941.

18. Schloss, G.: Schweiz. Ztschr. f. Path. u. Bact. **11**:109, 1948.

19. Hartroft, W. S., and Best, C. H.: Brit. M. J., **1**:423, 1949.

20. Bechgaard, P., and Bergstrand, A.: Acta endocrinol. **2**:61, 1949.

21. Selye, H.; Stone, H.; Nielsen, K., and Leblond, C. P.: Canad. M. A. J. **52**:571, 1945.

crude suspension.[22] It is worth noting that purified adrenocorticotropic hormone (ACTH) injected under the same experimental conditions in daily doses of 0.1 mg. for twenty-four days did not produce any trace of nephrosclerosis.[23]

Our present investigation shows that a crude suspension or an alkaline extract of anterior pituitary gland had similar nephrosclerotic potencies. We confirm Selye's[5] observation that extrarenal arteritis is seldom seen in animals so treated and also that the adrenal glands are hemorrhagic and necrotic, especially in rats with the most severe renal lesions.

It had been reported by Davson, Ball and Platt[23] and others (reviewed by the last authors) that lesions of clinical nephrosclerosis are sometimes difficult to distinguish from those of periarteritis of the kidney. Careful examination of the kidneys from our various experimental groups revealed only 1 specimen the condition of which could be described as renal periarteritis. This process occurred in a rat with both kidneys wrapped and with a blood pressure of 208 mm. of mercury on the twenty-eighth day, which persisted around this level until autopsy on the one hundred and ninth day; macroscopic examination revealed generalized mesenteric arteritis. Histologically, the kidney showed hemorrhages in the medulla, generalized dilatation of tubules with albuminous casts and focal necrosis of very few glomeruli but no definite interstitial nephritis. The vascular lesions consisted of focal necrotizing arteritis with cellular infiltration restricted to the intralobar and arcuate arteries. It is interesting to note that in both locations these lesions took place at points of branching (fig. 3). These changes are obviously not those of malignant nephrosclerosis but correspond more accurately to those of periarteritis because of their localization and the cellular infiltration.

The question arises whether nephrosclerosis is the cause or the result of vascular hypertension. The following observations would tend to support the view that the structural renal damage is secondary and a result of hypertension and that malignant nephrosclerosis is the advanced phase of a vascular disease which in earlier phases is relatively benign. 1. In rats with one kidney wrapped, vascular renal lesions predominate in the contralateral "normal" kidney. This has already been noted by others.[24] 2. In rats with both kidneys wrapped, characteristic lesions of nephrosclerosis appear during the chronic phase of hypertension. 3. In desoxycorticosterone-treated rats, hypertension appears at a time when vascular or severe parenchymatous renal lesions are not discernible. 4. Discontinuation of desoxycorticosterone treat-

22. Hay, E. C., and Seguin, P.: Am. J. Physiol. **147**:299, 1946.
23. Davson, J.; Ball, J., and Platt, R.: Quart. J. Med. **17**:175, 1945.
24. Schroeder and Neumann.[15b] Wilson and Byrom.[17] Schloss.[18]

ment is followed by a definite decrease in blood pressure but a persistence of nephrosclerotic lesions. Similar conclusions have been reached by Friedman and Friedman.[25] To these we must add the observation of Byrom and Dodson,[26] who showed that sudden strains placed on the arterial walls by repeated forced injections of Ringer's solution made into the carotid artery produced focal necrosis of the small arteries of the kidney. However, as these authors pointed out, other factors besides high blood pressure may be involved in the production of these lesions.

To turn now to a comparison of the lesions of the three experimental groups studied, nephrosclerotic lesions were found in every group with some differences in their incidence. Desoxycorticosterone was the most effective agent, eliciting nephrosclerosis in all the rats (in twenty-nine days with 5 mg. or fifty-three days with 2.5 mg. of desoxycorticosterone acetate per day); kidney wrapping was next, and finally factors of the anterior lobe of the pituitary gland, either in suspension or in an alkaline extract. The same conclusion could be drawn concerning the incidence of extrarenal vascular lesions: Desoxycorticosterone was the most and the pituitary preparations the least active. When examining tissues from the steroid-treated rats, one is struck by the severity, the diffusion and the uniformity of the lesions. There is a generalized chronic inflammatory reaction. It is first observed in the perivascular tissues of mesenteric arteries and arterioles, then in the renal parenchyma, the peripelvic tissues, the intestines, the heart, the meninges and the cerebral cortex. The lesions of arthritis reported by Selye and associates[11] should also be included in this list. Another pathologic feature of this hormonal action is a widespread deposition of fibrinoid material. These generalized pathologic effects obtained by prolonged administration of this steroid reflect its capacity to elicit a degenerative process already referred to as fibrinoid diathesis or hyalinosis (Timiras, Faribault and Selye[27]).

Though there is histologic identity between the mesenteric arterial lesions in the rat and periarteritis (polyarteritis) nodosa in the human patient, it cannot be claimed that the clinical disease has been reproduced. Because several types of necrotizing vascular lesions occur in man, it is necessary to define carefully the groups selected for comparison. Thus Zeek, Smith and Weeter[28] studied rat arteritis and its possible human equivalents. They found two well defined types in 31 human

25. Friedman, S. M., and Friedman, C. L.: Proc. Canad. Physiol. Soc., Montreal, 1949, p. 16.

26. Byrom, F. B., and Dodson, L. F.: J. Path. & Bact. **60**:357, 1948.

27. Timiras, P. S.; Faribault, C., and Selye, H.: Geriatrics **4**:225, 1949.

28. Zeek, P. M.; Smith, C. C., and Weeter, J. C.: Am. J. Path. **24**:889, 1948.

cases of necrotizing panarteritis, one associated with hypersensitivity and usually involving venules and arterioles as well as arteries, the other characteristic of periarteritis nodosa as commonly known, involving muscular arteries. The arterial changes in our animals, as in theirs, appear identical with those described in the latter group. However, there are other features of the disease in rats, such as the splanchnic localization of the vascular lesions, the frequent occurrence of renal changes identical with malignant nephrosclerosis and the constant presence of hypertension, which are not part of clinical periarteritis (polyarteritis) nodosa. It seems unlikely that the experimental disease of the rat is the same as the human disease syndrome; one can conclude only descriptively that the necrotizing mesenteric arterial lesions are identical. The majority of the experimental arterial lesions in rats are necrotic at the outset and involve all vessel coats. The term "necrotizing panarteritis" describes it most succinctly and avoids an untoward clinical connotation.

These vascular lesions have been ascribed by Wilson and Byrom [17] to the sudden strain placed on the blood vessel walls by a rapid rise in blood pressure. Smith, Zeek and McGuire [29] reported that the determining factors in the production of panarteritis were the local infection incident to silk wrapping and the high blood pressure. An increased incidence of lesions has also been noted by Cromartie [16] in rats with suppurative renal infection. However, in a subsequent paper, Smith and Zeek [30] were of the opinion that the organisms were not responsible per se in the production of the arterial lesions but that the pus pockets formed between the silk and the renal tissue increased the compression on the kidney and therefore the blood pressure. As to the role of blood pressure levels, their data [29] indicate that differences from 7 to 11 mm. of mercury between average blood pressure values of two groups were sufficient to elicit panarteritis in one group while none occurred in the other. In our experience such variations are insignificant; they are inherent in the method itself and in the variability of blood pressure levels, especially in hypertensive rats (Loomis [9]). Our data, as well as those of Loomis,[9] tend to show that although rats with a high blood pressure may be more susceptible to panarteritis, this is not a constant association. It should be mentioned that Byrom and Dodson [26] did not observe vascular lesions following repeated forced injections in any organ except the kidney. That other contributory factors which may be grouped in the term "fibrinoid diathesis" are involved is suggested by the following observation: Rats treated with desoxycorticosterone for twenty-nine days were found to have panarteritis although blood pres-

29. Smith, C. C.; Zeek, P. M., and McGuire, J.: Am. J. Path. **20**:721, 1944.
30. Smith, C. C., and Zeek, P. M.: Am. J. Path. **23**:147, 1947.

sure levels around 200 mm. of mercury existed for a short period; on the other hand, rats with wrapped kidneys and a similarly high blood pressure presented the same lesions only after a much longer time.

Goldblatt [31] expressed the opinion that hypertensive vascular lesions are the result of renal excretory failure. If we accept that a rise in blood nonprotein nitrogen or urea constitutes a satisfactory index of the uremic state, observations of Fasciolo and Cramer [32] and Friedman, Jarman and Klemperer [16] showed that normal blood urea values are compatible with the presence of vascular lesions. In the present study we determined blood urea nitrogen in a certain number of rats which had both kidneys wrapped and found that although in some animals with vascular lesions the levels ranged up to 100 mg. per hundred cubic centimeters, in others, equally diseased, the levels were within the normal range (10 to 20 mg.). Furthermore, experiments by Wilson and Byrom [17] on rats that had one kidney clamped, or those of Selye and Stone [33] on rats with "endocrine kidney," established that severe vascular lesions occur in rats with adequate renal excretory function. Although uremia is not a necessary factor for the production of these lesions, its role should not be overlooked. This is shown by observations on rats or dogs with both kidneys removed, ureters ligated or renal arteries occluded [34] and a more recent one on bilaterally nephrectomized dogs maintained alive by the use of the artificial kidney.[35] But hypertension was probably present in these experiments also.

Thus, in summary, the vascular lesions produced in rats by three different experimental procedures, each of which seems to have a different mechanism, are identical. The identity of tissue response suggests a final common pathway of arterial damage. The evidence at hand points to arterial hypertension as the most significant pathogenetic factor. If this is the case, the experimental lesions must be considered as representing hypertensive arterial and arteriolar disease in the rat. The fact that the renal lesions correspond exactly with those of malignant nephrosclerosis of human beings accords with this assumption. The disparity between the splanchnic arteritis of rats and the corresponding changes in human beings does not oppose the assumption if the patho-

31. Goldblatt, H.: J. Exper. Med. **67**:809, 1938.

32. Fasciolo, J. C., and Cramer, F. K.: Rev. Soc. argent. de biol. **14**:393, 1938.

33. Selye, H., and Stone, H.: J. Urol. **56**:399, 1946.

34. Winternitz, M. C.; Mylon, E.; Water, L. L., and Katzenstein, R.: Yale J. Biol. & Med. **12**:623, 1940. Holman, R. L.: Am. J. Path. **19**:147, 1943. Leiter, L., and Eichelberger, L.: J. Clin. Investigation **22**:11, 1943. Byrom and Dodson.[36]

35. Muirhead, E. E.; Vanatta, J., and Grollman, A.: Arch. Path. **48**:234, 1949.

logic processes observed in the rat are taken as expressing a response characteristic of that species. Lastly, this concept and its demonstration provide the experimentalist with a secure basis for studies directed toward the control of hypertensive arterial disease in human beings.

The lesions found in the kidneys, the heart, the splanchnic vessels, the brain and the subcutaneous tissues of rats made hypertensive by kidney wrapping or by injections of desoxycorticosterone acetate or of anterior pituitary preparations are compared and found to be essentially the same. They are most frequently reproduced with the desoxycorticosterone treatment.

The renal changes are identical with those of malignant nephrosclerosis of human beings, and the brain changes, with those described by Scheinker under the term of hypertensive arteriolopathy.

The most striking vascular lesion is a splanchnic panarteritis which seems to progress centripetally toward the lumen of the vessel. The myocardial lesions consist of parenchymal necrosis and fibrosis, and extensive perivascular proliferation accompanied by deposition of fibrinoid or necrosis.

In view of their diverse causes, all these pathologic changes are considered to be the manifestation of hypertensive vascular disease of the rat.

The desoxycorticosterone acetate (DOCA) used in this investigation was supplied by Dr. E. Henderson, of the Schering Corporation, Bloomfield, N. J.

SEQUENCES IN THE DEVELOPMENT OF CIRRHOSIS OF THE LIVER IN CASES OF ERYTHROBLASTOSIS FETALIS

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IN THE LITERATURE prior to 1934 there were a few scattered reports of cirrhosis occurring in children with severe and prolonged postnatal icterus.[1] The purpose of this study is to describe the development of cirrhosis of the liver as it is observed in cases of erythroblastosis fetalis. A special attempt was made to obtain a series illustrative of the transitional forms between minor necrosis and definite cirrhosis of the liver.

Hawksley and Lightwood [1f] in 1934 were the first to discuss the possibility that such cirrhosis depends etiologically on erythroblastosis fetalis. They presented the clinical and pathologic observations in 9 cases of erythroblastosis of the icterus gravis type. At postmortem examination, diffuse fibrosis was found about the polygonal cells and portal tracts of the liver in 7 cases and actual cirrhosis in 1. The fibrosis was predominantly lobular but was seldom pronounced when the postnatal survival of the patient was prolonged. Degenerative changes in the parenchymal cells of the liver were described as diminished staining reaction of the cytoplasm, vacuolation of cells and loss of cell outline. These changes, however, could not be differentiated from those caused by postmortem autolysis. The distinctive feature of liver cell necrosis, the polymorphonuclear cells infiltrating the substance of parenchymal cells, was mentioned once in the case of definite cirrhosis.

Following the Hawksley and Lightwood report in 1934, other investigators [2] discussed, especially in the English literature, a similar

From the departments of pathology of Children's Medical Center, Boston Lying-in Hospital and Harvard Medical School.

1. (*a*) Hoffmann, W., and Hausmann, M.: Monatsschr. f. Kinderh. **33**:193, 1926. (*b*) Pfannenstiel, J.: München. med. Wchnschr. **55**:2169, 1908. (*c*) de Lange, C.: Monatsschr. f. Kinderh. **14**:351, 1917-1918. (*d*) Bossert, O.: Ibid. **14**:174, 1916. (*e*) Curtiss, C. C.: Arch. Pediat. **51**:396, 1934. (f) Hawksley, J. C., and Lightwood, R.: Quart. J. Med. **3**:155, 1934.

2. (*a*) Braid, F., and Ebbs, J. H.: Arch. Dis. Childhood **12**:389, 1937. (*b*) Lightwood, R. C.: Ibid. **18**:156, 1943. (*c*) Lightwood, R., and Bodian, M.: Ibid. **21**:209, 1946. (*d*) Parsons, L. G.; Hawksley, J. C., and Gittins, R.: Ibid. **8**:159, 1933. (*e*) Gilmour, J. R.: Ibid. **19**:1, 1944. (*f*) Ferguson, J. A.: Am. J. Path. **7**:277, 1931.

causation for juvenile cirrhosis. In 1944 Gilmour [2e] described pathologic conditions of the liver in a series of 41 infants with various types of erythroblastosis: 13 with universal hydrops, 26 with icterus gravis and 2 with congenital anemia. In the group with universal hydrops the reticulum content of the liver was increased in 10 cases; in 2 of these there was also an increased collagen content. In 2 cases in the larger group with icterus gravis an increase of the reticulum content was demonstrated, amounting in 1 case to distinct fibrosis. A similar increase was not described among the pathologic conditions in either of the 2 cases of congenital anemia. Necrosis of the liver was not mentioned in the postmortem examination of the group with universal hydrops, but was reported twice in the group with icterus gravis as liver cells which "appeared necrotic." Concerning one of these 2 cases the author stated that there was a loss of liver cells in "submiliary foci." He suggested an Rh incompatibility as a possible etiologic factor but did not support this by any specific data.

Although the disturbance of the liver cells was recognized and reported in the American literature, pathologic changes in the liver were not distinctly described. Ferguson [2f] made no reference to parenchymal damage. Diamond, Blackfan and Baty [3] mentioned vacuolation of the liver cells and possible fibrosis of the livers in their group of cases with universal hydrops, but did not detail their findings. Sobel and Zucker [4] reported 1 instance of fibrosis of the liver in which nuclear damage of the brain was observed at postmortem examination. Potter [5] mentioned briefly the possibility of fibrosis of the liver in infants whose postnatal survival was of short duration. Rosenblatt,[6] reporting massive necrosis of the liver in 3 infants who had received replacement transfusions, considered the necrosis a toxic phenomenon caused by excessive sodium citrate or calcium gluconate, administered in the process of transfusion.

PRESENT STUDY

In this presentation a description of the usual pathologic changes occurring in the liver in erythroblastosis is given first, followed by a report and discussion of a representative case or cases from three groups of cases of erythroblastosis. The classification of these groups is based on the degree of liver degeneration observed at postmortem examination: group 1, definite necrosis of the liver; group 2, fibrosis and necrosis of the liver, and group 3, cirrhosis of the liver. In addition to a detailed

3. Diamond, L. I.; Blackfan, K. D., and Baty, J. M.: J. Pediat. **1**:269, 1932.

4. Sobel, I. P., and Zucker, J. M.: J. Pediat. **16**:445, 1940.

5. Potter, E. L.: Rh: Its Relation to Congenital Hemolytic Disease and to Intragroup Transfusion Reactions, Chicago, The Year Book Publishers, Inc., 1947.

6. Rosenblatt, P.: Am. J. Clin. Path. **18**:700, 1948.

discussion of the representative cases, other cases in each group will be briefly summarized in tables 1, 2 and 3.

MATERIAL AND METHODS

The material available was composed of clinical records, autopsy protocols and fixed tissues from 141 cases. All autopsies were performed at Children's Medical Center or at Boston Lying-in Hospital. In each case there was sufficient clinical and pathologic evidence for a diagnosis of erythroblastosis. No subdivision according to icterus gravis or fetal hydrops was made, because either type may be considered as a clinical stage determined by the severity and duration of the erythroblastotic process.

Sixteen cases were selected from the total series of 141 for special study. Liver sections were stained routinely with hematoxylin-eosin or eosin-methylene blue. The majority of these sections were also stained with Mallory's aniline blue and hemofuchsin technics, Perles' method for hemosiderin and Foot's modification of the Bielschowsky-Maresch method for reticulum. A few sections were studied for glycogen content by Best's carmine stain and for fat content by the scarlet red technic.

THE PATHOLOGIC CHANGES USUALLY OBSERVED IN THE LIVER IN ERYTHROBLASTOSIS FETALIS

Gross Description.—The liver may be enlarged slightly, or occasionally to three and a half times its normal size. The capsule is smooth, and the external surface has a purple-brown hue. Bile stasis is rarely seen on gross examination. The liver has a yellow-green sheen on the cut surface, although the underlying substance has a basic purple-brown color. The parenchyma may evert slightly on cutting; it is smooth, and the lobular markings are indistinct or absent. The surface is frequently bile stained, but there is no bile exudation on cutting. Extensive congestion may be present. The bile ducts are patent, and the gallbladder may contain only thin, almost colorless bile.

Microscopic Description.—The most characteristic feature is the erythropoietic cells which are present in the sinusoids. They appear in all stages of maturation when the erythroblastotic process has been severe. In the earliest stage there are proerythroblasts with large nuclei. These nuclei present prominent chromatin clumps and a sharp nuclear membrane and are surrounded by a thin rim of dark basophilic cytoplasm. In cross section the proerythroblasts are seen grouped in small foci of 5 to 10 cells with no adult cells in the immediate vicinity. During cellular maturation and proliferation, erythroblasts and normoblasts arise, and the number of cells in the foci increase. In the last stage the cells of the foci consist of normoblasts and normocytes with a sprinkling of adult red cells. The estimate of cellular activity must be based on the number of cells and the variation in cell maturation in a single focus, as it is difficult to demonstrate mitotic figures in the postmortem liver section.

Myelopoietic cells are not present in the parenchymal sinusoidal areas but predominate in the periportal connective tissue. In the latter area a few erythroid elements may also be found. No rigid correlation exists between the activity of the erythroid cells in the lobules and that of the myeloid cells in the portal areas. The former, in a liver in a case in which the postnatal survival of the patient is prolonged, may show intense activity, yet the myeloid cells in the periportal tissue may exhibit little or no activity. When cellular activity is minor or regressing, remnants of the erythroid maturation chain seem to be discernible at both ends of the series. These remnants are small clusters of normoblasts and normocytes that have remained along with erythroblasts and proerythroblasts, appearing singly or in groups of two or three. Extruded nuclear fragments may be seen occasionally in the sinusoids.

Late in the process of cell maturation the portal areas contain only a few eosinophilic polymorphonuclear cells. The regular distribution of connective tissue about the portal triads may appear more prominent than usual owing to the separation of the constituent fibers. The entire region may seem widened by the "infiltrating" hematogenous tissue. An edematous change is encountered in the lobular areas where there is no increase in fibrous or reticulum tissue. The reticulum fibers stain less sharply and densely and are loosely arranged; they always skirt the erythropoietic foci and do not ramify within their substance. This is in sharp contrast to the close relation in the portal triads, where the hemopoietic cells appear singly or in groups within the reticulum and fibrous tissue.

When erythropoiesis is widespread and active, the parenchymal cells shrink and occupy only a fraction of the usual area, and the sinusoidal spaces, filled either by red cells or erythropoietic tissue, are greatly dilated. The liver cells separated into groups of 2 to 3 cells are isolated from the rest of the cell column, which is broken and distorted. The nuclei are prominent unless overlying pigments of cytoplasm obscure them. Likewise, the nuclear membrane is prominent, and the chromatin clumps are large; the cytoplasm may be somewhat acidophilic and granular. Occasionally, large amounts of glycogen are demonstrable. More frequently, moderate amounts of fat are stored in small, irregular vacuoles of cytoplasm.

Pigment may be difficult to find in some instances, but it is usually a prominent feature in the cytoplasm of the parenchymal cells. In the majority of cases a bright yellow, granular pigment mass lies between the nucleus and the canaliculi of the parenchymal cells. This pigment never completely surrounds the nucleus when the cell is intact but may spread laterally to cover nearly half the cell. In sections stained for iron the iron-positive material in the parenchymal cells has the same distribution as the granular pigment. The iron is heavily deposited toward the

periphery of the lobules, and the maximum amounts are found in those cells adjacent to the portal areas. When the same sections are stained for hemofuscin with Mallory's basic fuchsin stain, an irregular distribution of the hemofuscin, similar to that of the iron, is observed in the same area; the distribution is rarely abundant. Iron-positive material is also found in the Kupffer cells, but here, in contrast to that deposited in the parenchymal cells, it occurs in small amounts as ill defined fine particles and occupies the entire cell cytoplasm, often obscuring the nucleus. It tends to be more heavily deposited in the reticuloendothelial cells toward the periphery of the lobule. Many Kupffer cells do not contain iron deposits even when the parenchymal cells are heavily impregnated. In a few instances the iron deposited in the sinusoidal phagocytes in the liver and the spleen approaches the levels of that in the polygonal liver cells. In cases in which the postnatal survival of the patient is more protracted, the distribution of iron gradually shifts from the parenchymal cells to the Kupffer cells, and toward a greater concentration at the periphery of the lobule. The macrophages in the portal areas apparently absorb large amounts of iron. Iron also may be demonstrated in the epithelial cells of the bile ducts; curiously, it lies below the nucleus away from the lumen. In Kupffer cells, the bile, unlike the iron, is more frequently found in those toward the centers of the lobules and is more common in the presence of parenchymal necrosis; thus it is also recognizable within the cell cytoplasm.

Bile pigment is rarely seen in an intact parenchymal cell. The canaliculi almost without exception contain bile thrombi, which may not be easily discerned in the paraffin sections, because the majority of them are extracted by the lipid solvents. However, they are easily demonstrated in frozen sections stained with methylene blue. In those cases in which the postnatal survival of the patient is short, the intracanalicular thrombi do not maintain any constant position; when the postnatal survival is more prolonged, they remain longest in the central areas. In the small intrahepatic bile ducts bile thrombi appear frequently but inconstantly and do not seem directly related to the severity of the erythroblastotic process.

It should be emphasized that many of the aforementioned pathologic features, such as iron deposits, bile thrombi, small foci of erythropoiesis, glycogen and fat vacuoles, are not specific in erythroblastosis but can be found in different degrees in the livers of infants whose death was not caused by erythroblastosis.

GROUP 1: CASES OF NECROSIS OF THE LIVER IN ERYTHROBLASTOSIS FETALIS

Group 1 is composed of those cases (table 1) in which at postmortem examination there was damage of the liver, histologically verifiable, with definite parenchymal necrosis but with no increase of reticulum or

fibrous tissue. Ten cases out of the total series of 141 belonged in this group. In 6 of these 10 cases an investigation of the antigenic situation in the mother's, the father's and the infant's blood furnished sufficient evidence for the diagnosis of erythroblastosis. In 3 other cases there was a family history of siblings dying from erythroblastosis, the diagnosis being based on the clinical history or on postmortem examination. There was only 1 case (6) in which neither the investigation of the Rh blood factor nor the family history contributed to a diagnosis of erythroblastosis. In all cases in group 1 the clinical course and the postmortem observations were consistent with those seen in erythroblastosis. The morphologic aspect of the placenta furnished additional evidence in a few cases. All serologic tests for syphilis were negative.

CASE 1.—The mother of the patient was 28 years old. Her blood was free of any Rh factor. She had previously borne two children. The first child was normal and the second, a product of the pregnancy immediately preceding that of which the patient was the offspring, was stillborn. The father's blood contained homozygous Rh. In the third pregnancy the mother came to the hospital three weeks before term because of a rising anti-Rh agglutinin titer of 1:512. She delivered at cesarean section an infant of normal appearance who weighed 6 pounds, 2 ounces (2,778 Gm.) and cried lustily. Anti-Rh saline and blocking antibodies were found in the blood; the placenta was not enlarged and exhibited no evidence of erythroblastosis.[7] A transfusion was performed, 480 cc. of the infant's blood being withdrawn and replaced with 540 cc. of Rh-free blood. After the transfusion the infant became increasingly pale and jaundiced. Clinical signs of kernicterus[8] developed, and the infant died on the second day of life. At postmortem examination diffuse petechial hemorrhages were found in the skin and the viscera. The lungs were overweight, massively congested, and exhibited a large amount of intra-alveolar hemorrhage. The spleen weighed 13 Gm. (normal weight of spleen of a newborn infant, 7 Gm.[9]). The liver weighed 122 Gm. (normal, 78 Gm.). No other abnormalities were noted in the liver. The extrahepatic ducts were patent and normal in form. Kernicterus was not evident grossly or microscopically.

Microscopic Appearance of Liver.—The erythropoietic centers in the liver were not prominent but manifested all stages of red cell development; the larger centers contained the higher proportion of more adult cells. The foci were not confluent but scattered in the parenchyma. Myeloid elements, particularly eosinophilic elements, were conspicuous in the portal tracts. The majority of the parenchymal cells were full, not shrunken, and were regularly arranged into cell columns. A few small fat vacuoles were present in scattered cells, and occasionally the parenchymal cells contained small amounts of bile. Bile stasis was an outstanding feature; practically every bile canaliculus contained either solid plugs or small globules of bile. The bile ducts were almost free of bile pigment. Widespread areas of midzonal necrosis (fig. 1) extending at times from one lobular area to another were sharply outlined. The necrotic parenchymal cells seemed swollen and had a uniformly bright eosinophilic granular appearance. There was loss or pyknosis of the nuclei; most of the cell outlines were retained. Polymorphonuclear cells

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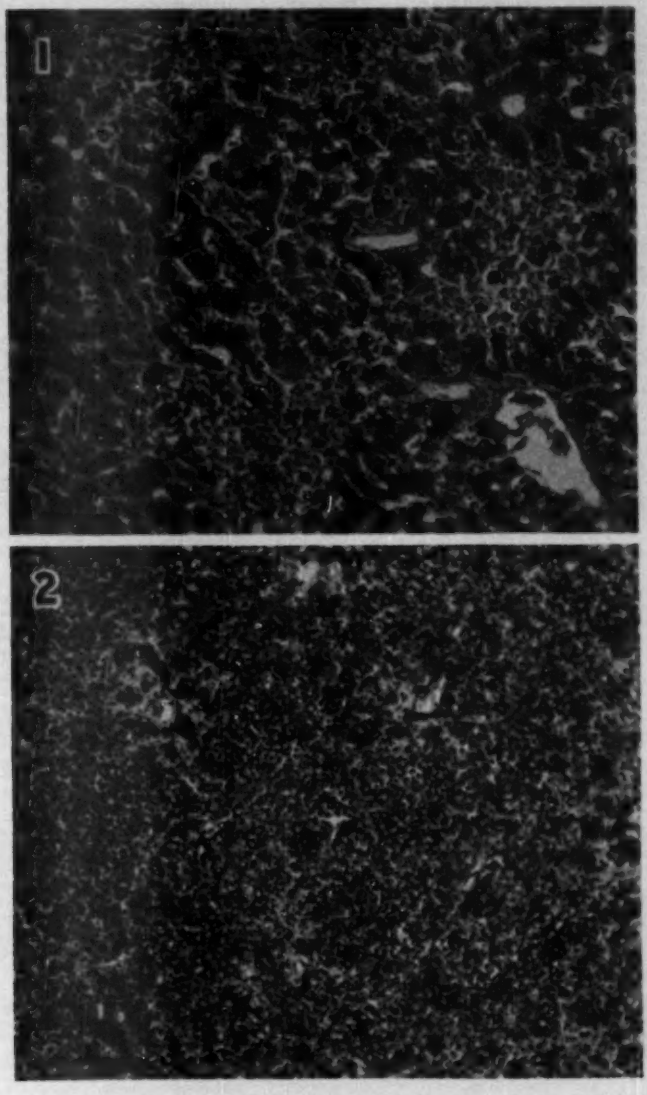


Fig. 1 (group 1, case 1).—Photomicrograph of liver in which the multilobular midzonal necrosis is clearly outlined. The erythropoietic centers are not prominent. (Eosin-methylene blue; × 150.)

Fig. 2 (group 1, case 4).—Photomicrograph of liver showing areas of necrosis between the intact parenchyma of the center and the periphery of the lobule. Massive erythropoiesis is present. (Eosin-methylene blue; × 150.)

TABLE 1.—*Cases of Erythroblastosis with Necrosis of the Liver*

Case,* Year, Age of Infant	Family History	Clinical History	Rh Factor	Gross and General Pathologic Observations	Microscopic Observations of Liver
1 1947 2 days	Mother 28 year old para III, grav. III. Offspring of previous pregnancy stillborn. Present pregnancy terminated by cesarean section	Infant given replacement transfusions; increasing jaundice developed; clinical kernicterus	Mother's blood Rh free. Infant's blood contained Rh. Father's blood contained homozygous Rh	Massive pulmonary congestion with intra-alveolar hemorrhage. Spleen, 13 Gm. Liver, 122 Gm. No kernicterus. Placenta immature	Moderate erythropoiesis; prominent bile stasis; sharply outlined midzonal necrosis with polymorphonuclear infiltration (fig. 1)
2 1943 ½ hr.	Mother 36 year old para IV, grav. IV. Offspring of previous pregnancy, erythroblastosis; autopsy	Infant edematous; lived ½ hour; no transfusions	Not examined	Pulmonary hemorrhage; aspiration of amniotic sac contents. Ascites, 60 cc. Spleen, 50 Gm. Liver, 200 Gm. No kernicterus. Erythroblastotic placenta, 1,320 Gm.	Moderate erythropoiesis; small focal areas of midzonal necrosis with polymorphonuclear infiltration
3 1941 1 day	Mother 34 year old para II, grav. II. 1 erythroblastotic infant delivered subsequently	Weighed 8 lb. (3,628 Gm.) but premature by dates; jaundiced at birth; anemic; clinical kernicterus; transfusion with 30 cc. of father's blood	Not examined	Pulmonary aspiration of amniotic sac contents; small areas of aspiration pneumonia. Spleen, 12 Gm. Liver, 110 Gm. Kernicterus. Erythroblastotic placenta, 2,000 Gm.	Moderate erythropoiesis; occasional small, scattered areas of hyaline midzonal necrosis; rare polymorphonuclear invasion of parenchymal cells
4 1946 2 days	Mother 31 year old para II, grav. II. No maternal anti-Rh immune bodies 2 mo. before term	Infant born prematurely at 35 weeks; pale, icteric, edematous, with rapid respirations; given large transfusions with temporary improvement; clinical kernicterus	Mother's blood Rh free. Father's and child's blood contained Rh. Chord blood anti-Rh immune titer 1:32	Massive intra-alveolar pulmonary hemorrhage. Spleen, 20 Gm. Liver, 165 Gm. Kernicterus. Large erythroblastotic placenta	Massive erythropoiesis; prominent bile stasis; sharply outlined midzonal necrosis with polymorphonuclear infiltration (fig. 2)
5 1935 7 days	Mother para III, grav. III. 2d child anemic at birth; transfusion; jaundiced with enlarged liver at 4 mo.	Normal at birth; on 3d day jaundice, apathy; 7th day moribund; erythrocytes, 1,500,000; two transfusions	Not examined	Pulmonary aspiration of amniotic sac contents with minimal pneumonitis. Ascites, 100 cc. Spleen, 30 Gm. Liver, 175 Gm. Cut surface has areas of yellow-brown bordered by red. No kernicterus	Moderate erythropoiesis; massive midzonal and multilobular necrosis with much polymorphonuclear invasion
6 1941 2 days	Mother para II, grav. II. 1 sibling living and well	Normal at birth, then jaundice developed; clinical kernicterus; 6,000 nucleated red cells per cu. mm. of blood on day of death; given three transfusions from father	Not examined	Pulmonary aspiration of amniotic sac contents. Spleen, 26 Gm. Liver, 136 Gm., with areas of yellow-orange in parenchyma bordered by red line (fig. 3). Kernicterus	Massive, sharply outlined midzonal and multilobular necrosis; many areas free from necrosis; increased fat in necrotic areas; occasional infiltrating polymorphonuclears
7 1941 1 day	Mother 35 year old para III, grav. III; history of adequately treated syphilis; no history of stillbirths. 3 siblings well	Infant difficult to resuscitate; jaundice developed 5 hr. after birth; clinical kernicterus; given transfusion terminally	Mother's blood Rh free; no saline-Rh immune titer. Father's blood contained Rh. Infant's blood contained Rh; Hinton test negative	Pulmonary aspiration of amniotic sac contents with minimal pneumonia. Ascites, 20 cc. Spleen, 40 Gm. Liver, 160 Gm. No evidence of syphilis. No kernicterus	Active erythropoiesis; numerous small areas of midzonal necrosis; many invading polymorphonuclears in parenchymal cells
8 1942 2 days	Mother para II, grav. III. 1 sibling jaundiced at birth	Became jaundiced soon after birth; clinical kernicterus developed; 560,000 nucleated red cells	Mother's blood Rh free; no anti-Rh immune titer. Father's and infant's blood contained Rh	Minimal bronchopneumonia. Spleen, 40 Gm. Liver, 150 Gm. Kernicterus. Postmortem lung culture, sterile	Small focal areas of midzonal hyaline necrosis; invading polymorphonuclear cells; active erythropoiesis
9 1942 2 days	Mother para IV, grav. IV; received transfusion of husband's blood at first delivery for postpartum shock. 2d child erythroblastotic with recovery	Delivered after induction 1 week from term; rapidly increasing jaundice and edema; erythrocytes, 1,500,000; 190 nucleated red cells to 100 white cells	Mother's blood Rh free. Father's contained heterozygous Rh. No anti-Rh immune titer of chord blood	Pulmonary edema. Spleen, 30 Gm. Liver, 172 Gm. No kernicterus. Placenta large and boggy	Massive midzonal and multilobular necrosis; occasional invading polymorphonuclear cell; active erythropoiesis
10 1945 2 days	Mother para III, grav. III. 2 siblings jaundiced at birth and remained so for 2 weeks	1 mo. premature; jaundice noted 4 hr. after birth; clinical kernicterus developed; erythrocytes, 1,500,000; repeated transfusions	Mother's blood Rh free. Father's and infant's blood contained Rh	Pulmonary aspiration of amniotic sac contents; minimal aspiration pneumonia. Spleen, 12 Gm. Liver, 86 Gm. Areas of yellow-orange parenchymal discoloration. Kernicterus	Moderately large areas of sharply outlined midzonal necrosis; polymorphonuclear infiltration of parenchymal cells; suggestive areas with removal of parenchymal cells; active erythropoiesis

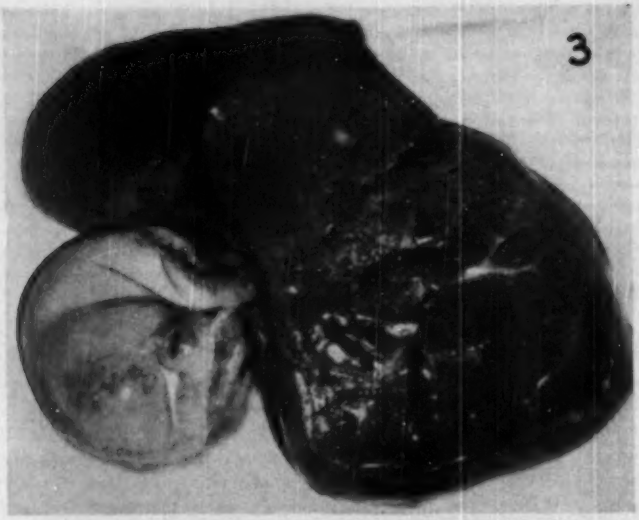
* Cases 1 to 4, inclusive, were taken from the records of Boston Lying-in Hospital; cases 5 to 10, inclusive, from the records of Children's Medical Center.

could be found within the necrotic cytoplasm of the parenchymal cells. There was no vacuolation of the necrotic cells. At the outer boundary of the coagulative necrosis there was a clear border area where the parenchymal cells had apparently been removed. Here the Kupffer cells were prominent and swollen with bile and iron pigment. Because of this narrow zone, collapse of the reticulum was not apparent. The necrotic areas containing well preserved erythropoietic elements took an intense fuchsinophilic stain with Mallory's aniline blue method. A slight prominence of the reticulum fibers was observed in the centers of the areas. There was no collapse of the reticulum framework. Iron stains revealed an absence of iron-containing material in the centers of the necrotic areas, but elsewhere the parenchymal cells contained moderate to large amounts of iron. There was a slight increase in the diffuse blue-staining reaction of the Kupffer cells bordering on the necrotic areas. The centers of the necrotic areas also contained a few red cells. No congestion was noted in the border areas.

Comment on Group 1.—What was the genesis of the necrosis in the livers in group 1? Was it due to factors unassociated with erythroblastosis? Are there other possibilities? Does the necrosis represent abscesses? The majority of these infants did not live long enough for omphalitis to develop, which is the most common source of hepatic abscess in the newborn. This process usually reaches its height near the end of the first week. The signs of infection precede those of jaundice. Infection of the umbilical vein was suspected grossly in case 7, especially after areas of discoloration were seen in the liver (fig. 3), but both the microscopic sections of the umbilical vein and the postmortem cultures failed to substantiate this suspicion.

Were the lungs the source of a bacterial inflammatory process in the liver? There was little evidence to support this possibility. Of the 8 patients in the group who had aspirated small but significant amounts of amniotic sac contents, only 4 exhibited any reaction to this foreign material. In some instances the reaction was characterized by the presence of polymorphonuclear cells along with the vernix and squamous cells in the alveoli. In case 8 there was microscopic evidence of bronchopneumonia with fibrin and polymorphonuclear infiltration of the alveolar walls and interstitial tissue. In cases in which an obvious reaction has been induced by aspirated material, as in this case of definite pneumonia, the postmortem cultures may be sterile. At other times, although there may be no obvious reaction due to the presence of aspirated material, the postmortem cultures may be positive. For example, in case 10 the postmortem cultures were positive for Bacillus coli and Staphylococcus albus. Possible sources of infection of the newborn, such as the kidneys and the upper respiratory passages, were found to be free of infection.

The histologic changes in the liver suggested abscesses, but the primary involvement of the liver cells, with necrotic change, and the secondary infiltrations of inflammatory cells were more consistent with primary necrosis. Neither bacteria nor forms suggestive of bacteria



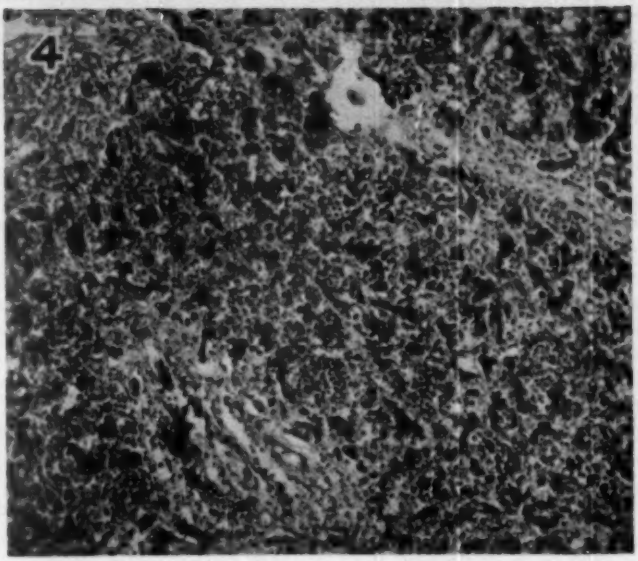


Fig. 3 (group 1, case 6).—Gross photograph of the liver and the attached stomach. The multilobular small areas of yellow discoloration are apparent in the center of the liver.

Fig. 4 (group 2, case 11).—Photomicrograph of liver showing periportal and lobular fibrosis with proliferation of bile ducts. Three portal triads are present in the photomicrograph. Erythropoiesis is active in the parenchyma, with many myeloid elements in the portal triads. (Eosin–methylene blue; × 150.)

were visible in any of the sections; specific stains for bacteria were not made. Since there was no evidence of infection metastasizing to other organ systems, it was difficult to associate these histologic changes with abscess formations.

Do these changes represent necroses from simple anoxemia combined with a possible toxic reaction to infection? Such a lesion is usually symmetric, diffuse and central in location; furthermore, the cells usually do not exhibit a coagulative type of necrosis.

Rosenblatt,[6] presenting 3 cases of massive hepatic necrosis occurring in erythroblastotic infants who had received exchange transfusions, considered the toxic effects of large doses of sodium citrate or calcium gluconate administered incident to the transfusion as the probable source of the necrosis. Three of the 10 patients in group 1 did not receive transfusions, yet their liver damage was indistinguishable in extent from that of patients who had been given repeated transfusions. Transfusions were given to 2 other patients terminally or in such small amounts that they were of no significance in the production of the lesions observed.

Definite ischemia was present in many of the hyaline, necrotic centers. No red cells were observed, and the sinusoids were collapsed by the swollen parenchymal cells. Apparently the ischemic process was not causative, since the sinusoids were normally dilated, some with congestion, in the areas proximal to the necroses. The iron stains revealed that the mineral content of the parenchymal cells was sharply reduced in the necrotic areas as compared with that in the surrounding zones. A similar reduction of pigment was observed in the sections stained for hemofuscin. The Kupffer cells in the boundary areas not only were more prominent but also contained more iron than the other cells in the surrounding regions. Evidence of deficient function also extended to the bile canaliculi, which lacked bile in the necrotic areas, although the Kupffer cells in areas bordering the necroses at times contained an increased amount of bile and iron pigment. Fat, the only element appearing more prominently in the necrotic areas than in healthy parenchyma, occurred in irregular and diffuse forms and not in discrete globules.

GROUP 2: CASES OF NECROSIS AND FIBROSIS OF THE LIVER IN ERYTHROBLASTOSIS FETALIS

Group 2 is composed of those cases in which at postmortem examination there was an obvious and significant increase of the reticulum and fibrous tissues of the liver (table 2). There were some cases of parenchymal necrosis. Rh incompatibility was not detected in this group, since all 4 cases occurred before the discovery of the Rh factor. However, in each case the family history was consistent with a diagnosis of erythroblastosis due to Rh incompatibility. In 3 cases this diagnosis

was confirmed by the postmortem examination of other siblings. Erythroblastosis was substantiated in 2 cases by a high count of nucleated cells of the erythrocytic series and anemia, although the period of postnatal survival was short. Generally the postmortem observations confirmed the diagnosis of erythroblastosis, especially the pathologic changes of the placenta in 3 cases.

Case 12.—This infant was delivered of a mother who had borne six children previously. The first four children were living and well and had had no recorded

Table 2.—*Cases of Erythroblastosis with Fibrosis and Necrosis of the Liver*

Case,* Year, Age of Infant	Family History	Clinical History	Rh Factor	Gross and General Pathologic Observations	Microscopic Observations of Liver
11 1932 ½ hr.	Mother para IV. 2 living children. 1 erythroblastotic infant; autopsy	Delivered by cesarean section; anemia; 297,000 red cells per cu. mm. of blood. Lived ½ hr.	Not examined	Lungs clear. Ascites, 50 cc. Spleen, 50 Gm. Liver, 300 Gm. Placenta large. Brain not examined	Erythropoiesis; bile stasis in canaliculi and parenchymal cells; diffuse increase in lobular reticulum; midzonal hyaline necrosis; portal fibrosis with bile duct proliferation (fig. 4)
12 1935 Few minutes	Mother para VII. 5th stillborn. First 4 children living and well. 6th macerated, stillborn; erythroblastotic placenta, 1,300 Gm. 1 erythroblastotic infant delivered subsequent to 7th child	Delivered at 38th week; yellow vernix; obstruction to breathing, with intercostal retraction	Not examined	Defect in bronchial cartilages; lungs atelectatic. Ascites. Spleen, 120 Gm. Liver, 190 Gm.; dark olive green. Placenta, 1,300 Gm. No kernicterus	Active erythropoiesis; canalicular bile stasis; parenchymal necrosis; increase in lobular reticulum; portal and lobular fibrosis with bile duct proliferation (fig. 5)
13 1937 Stillborn	Mother 42 year old para XII. 8 normal children. 9th stillborn. 10th jaundiced and edematous, stillborn. 11th lived 48 hr.; autopsy; erythroblastosis	Delivery complicated by hemorrhage; stillborn; 7 lb. 9 oz. (3,430 Gm.); not macerated	Not examined	Generalized edema. Ascites, 300 cc. Spleen, 88 Gm. Liver, 300 Gm. No kernicterus. Placenta, 1,300 Gm., erythroblastotic	Confluent erythropoiesis; canalicular bile stasis; much diffuse parenchymal necrosis; rare infiltrating polymorphonuclears; diffuse lobular and portal fibrosis; bile duct proliferation (fig. 6)
14 1939 2 days	Mother para IV. First 2 children normal at birth. 3d died with jaundice, first day of life	Infant hypotonic, cyanotic, moribund on admission; erythrocytes, 2,300,000; nucleated red cells, 300,000 per cu. mm. of blood; amniotic fluid, yellow	Not examined	Pneumonia; extensive pulmonary hemorrhage. Ascites, 25 cc. Spleen, 60 Gm., yellow-red discoloration to splenic capsule. Liver, 196 Gm.; green-brown. Kernicterus	Active erythropoiesis; canalicular bile stasis; occasional foci of necrosis; rare polymorphonuclear infiltration; lobular and portal fibrosis (fig. 7); bile duct proliferation

* Cases 11, 12 and 13 were taken from the records of Boston Lying-in Hospital; case 14, from the records of Children's Medical Center.

postnatal abnormalities. The fifth and sixth infants had been stillborn. The sixth was macerated, but the placenta weighed over 1,300 Gm. and exhibited changes associated with erythroblastosis. Nucleated cells of the erythrocytic series were numerous in the fetal vessels. The seventh pregnancy ended in the thirty-eighth week when the membranes ruptured prematurely with escape of deep yellow vernix. The delivery was uneventful. At birth the infant made vigorous respiratory efforts and had intercostal and subcostal retraction, but no satisfactory air exchange was established. Liver and spleen were palpable and huge. This infant lived only a few minutes. Two years later the mother again delivered a stillborn child. At postmortem examination both liver and spleen were enlarged. Changes due to

erythroblastosis were found in the placenta. Generalized edema and ascites were present. Grossly the lungs were atelectatic. The malformation of the cartilaginous rings of the main bronchus of the left lung gave a slitlike cross section to the bronchial lumen. Microscopically, the lungs were unexpanded and edematous and, like the other viscera, showed active erythropoiesis. The spleen and the liver weighed 120 Gm. and 190 Gm., respectively. The liver had a dark olive green color but otherwise was normal. The external bile ducts were patent and normally formed. The large adrenal glands weighed together 14 Gm.; the increased weight was due to the enlarged fetal cortex. No kernicterus was observed.

Microscopic Appearance of Liver.—There was moderately intense erythropoietic activity in the liver, evidenced by the presence of 10 to 12 centers per low power field. The majority of the centers were moderate in size and composed primarily of erythroblasts. The intralobular structure was considerably distorted by erythropoietic foci but was even more distorted by an intense and diffuse fibrous reaction (fig. 5), visible throughout the lobule but more pronounced near the portal areas. The reticulum stains showed partial collapse of the reticulum with an increase in the number and the width of the constituent fibers. A few young fibroblasts were recognized in the lobular areas. The aniline blue stain demonstrated a large amount of collagen surrounding the parenchymal cells which were changed by degeneration and regeneration. In large numbers of parenchymal cells the cell membrane was broken, bile droplets were diffused and the nucleus was karyorrhectic and displaced to the edge of the cell. The cytoplasm lacked its normal tinctorial quality because it was broken into large granular masses. Only a few infiltrating polymorphonuclear cells could be seen. The infrequently appearing multinucleate cell, found where the nuclei were piled closely, gave evidence of regeneration. Bile stasis in the canaliculi was prominent where the liver cells were intact. The smaller bile ducts contained bile, but the larger ones did not. The Kupffer cells were swollen with bile and occasionally contained phagocytosed erythrocytes. There was active proliferation of the bile ducts in the portal areas; the newly formed cell columns invaded the border zones of the lobules. The portal areas were widened, and the connective tissue was increased. Myelopoietic activity was moderate in these areas. A minimal amount of iron was present in the parenchymal and Kupffer cells.

Comment on Group 2.—In this group the observations concerning hepatic damage and disturbance differed in several respects from those in group 1. First, in 3 cases of group 2 (11, 12 and 13) the necroses were more diffuse and did not always have the hyaline refractile coagulative pattern. They were not as sharply outlined and did not cause as much polymorphonuclear reaction. The necrotic cells were slightly swollen, and the cytoplasm was granular. Bile droplets were diffused throughout the cells which had marginal nuclei that were faded and karyorrhectic. Instead of involving several liver cell columns simultaneously in a large area, the necrotic process appeared limited to a small group of cells within a single cell column. Second, bile stasis of the parenchymal cells was more prominent, although the canaliculi were also filled with bile. The bile stasis in the ducts was not significant in either group. Third, in each case the deposition of iron was far less than that in group 1. Parenchymal cells containing large quantities of iron were rare and scattered.

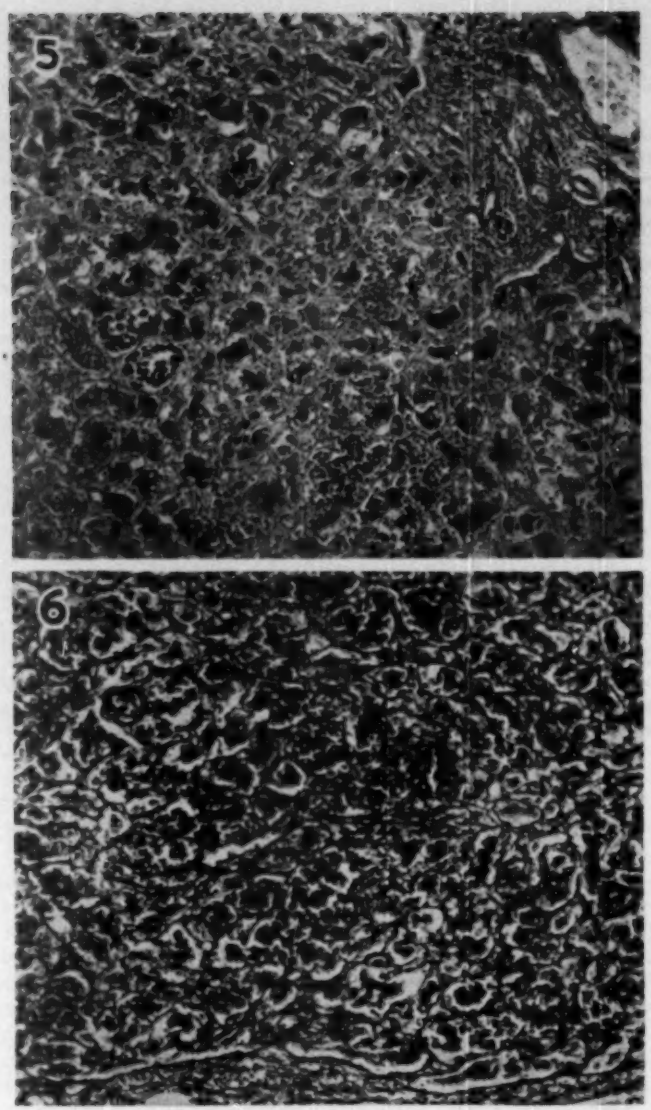


Fig. 5 (group 2, case 12).—Photomicrograph of liver showing diffuse fibrosis. The fibrous tissue isolates small groups of liver cells but has not caused distortion of the major lobular structure. (Mallory's aniline blue; × 150.)

Fig. 6 (group 2, case 13).—Photomicrograph of liver. The reticulum fibers surround but do not pass through the erythropoietic centers. The fibers are increased in width and number. (Foot's reticulum stain; × 150.)

Iron deposition was small in the Kupffer cells. The decrease in the amount of iron in the parenchymal cells might have been related to a longer period of disturbance of the liver than was encountered in the previous group. If the parenchymal cells were functioning at a low physiologic level, they might fail to absorb iron from the sinusoids. The same disturbances might account for the increase of bile in the parenchyma. The regeneration of the polygonal cells was consistent with the longer duration of the destructive process. Most of the damage in group 1 was too recent for much regeneration to have occurred.

The diffuse lobular distribution of the fibrosis in group 2 was consistent with the repair of a process observed in the more acute lesions in the previous group. An unevenness in the distribution of the fibrosis was detected, especially in case 14. Although every lobule was involved to some degree, there were distinct variations in the intensity of the fibrosis. The general lobular structure was always preserved; regeneration was focal in character rather than lobular and was most active along the periphery of the lobule. In spite of the widespread active fibrosis and the break-up of liver cord arrangements, the general liver patterns were preserved. Henderson[10] reported fibrosis of the liver in a group of macerated stillborn infants with congenital hydrops, but the photomicrographs accompanying the report were of such poorly preserved tissues that it was difficult to accept conclusions based on them.

With the exception of case 14, in which pneumonia was also found, a bacteriologic cause of the hepatic lesions was dismissed. In the case mentioned the hepatic damage definitely antedated the pneumonia.

GROUP 3: CASES OF CIRRHOSIS OF THE LIVER IN ERYTHROBLASTOSIS FETALIS

Group 3 is composed of the only 2 cases (table 3) in which at postmortem examination there was definite cirrhosis of the liver with actual distortion of the portal triad–lobular arrangements. In both cases, which are reported in detail, the fully developed cirrhosis occurred early in postnatal life.

CASE 15.—This child was born of a mother whose blood was free of any Rh factor and who previously had given birth to three children, all of whom were living and well. Both the father and the infant under discussion had an Rh factor in their blood. At the time of delivery the mother had a low anti-Rh agglutinin titer. The amniotic fluid contained meconium, and the vernix was stained yellow. Grossly, the placenta was normal. On admission to the hospital at 20 hours of age the child appeared cyanotic, somnolent and limp. His eyelids were edematous, and his color copper yellow. The liver and the spleen were palpable below the costal margin for a distance of 3½ fingerbreadths and 1½ fingerbreadths, respectively. The Moro and grasp reflexes were depressed. At initial examination of the blood the erythrocytes numbered 1,700,000 per cubic millimeter of blood and

10. Henderson, J. L.: Arch. Dis. Childhood **17**:49, 1942.

the hemoglobin level was 30 per cent of normal. Nucleated cells of the erythrocytic series comprised one half the nucleated cells in the peripheral blood. For one month the infant was given multiple transfusions of Rh-free blood with only partial success in restoring and maintaining the erythrocyte count and hemoglobin level. The erythrocyte count was finally stabilized between 2,500,000 and 3,000,000 per cubic millimeter of blood and the hemoglobin level at 50 per cent of normal. The jaundice continued unabated, although the size of the liver and the spleen became somewhat diminished. The stools contained bile. The urobilin level of the urine was normal. At 1 month of age the patient was transferred to a convalescent home, where diarrhea developed ten days prior to death. The liver and the spleen were still palpable, and the intense yellow-green jaundice remained until the infant died, three days after being readmitted to the hospital.

At postmortem examination a well developed but poorly nourished boy was observed. Generalized icterus was visible. Grossly the heart and the lungs were

TABLE 3.—*Cases of Erythroblastosis with Cirrhosis of the Liver*

Case,* Year, Age of Infant	Family History	Clinical History	Rh Factor	Gross and General Pathologic Observations	Microscopic Observations of Liver
15 1944 6 weeks	Mother para IV; 3 previous pregnancies normal	Yellow vernix. Infant became jaundiced at 1 day; copper-yellow at 20 hr.; repeated transfusions; anemia; many nucleated red cell forms; clinical kernicterus	Mother's blood Rh free; low anti-Rh titer at delivery. Father's and infant's blood contained Rh	Generalized icterus. Chronic pneumonitis. Spleen, 46 Gm. Liver, 182 Gm.; dark green. No kernicterus. Placenta normal grossly	Slight erythropoietic activity; cirrhosis; active parenchymal necrosis and regeneration; bile stasis in canaliculi and liver cells (fig. 8)
16 1939 2 weeks	Mother para IV, grav. III. Subsequently delivered a 2d pair of twins; 1 stillborn, other required repeated transfusions; erythroblastotic placenta	Infant jaundiced at birth; increased jaundice on 5th day; anemia; erythrocytes, 2,000,000; 1,000 nucleated red cells per cu. mm. of blood on 5th day; repeated transfusions	Mother's blood Rh free; high anti-Rh agglutinin titer with subsequent pregnancy. Father's blood contained Rh	Diffuse interstitial pneumonia, chronic pneumonitis. Liver and spleen together weigh 198 Gm. Liver, firm, green, nodular (fig. 9). Bile ducts patent. No kernicterus	Slight erythropoiesis; cirrhosis; active necrosis and regeneration of parenchyma (fig. 10); parenchymal bile stasis

* Cases 15 and 16 were taken from the records of Children's Medical Center.

normal, but microscopically there was diffuse chronic pneumonitis with many macrophages, swollen lining epithelial cells and a few intra-alveolar remnants of squamous cells. The spleen weighed 46 Gm. (normal weight for this age, 13 Gm.). It was normal in shape and firm in consistency, and on cut section the malpighian bodies were prominent. The liver weighed 182 Gm. (normal, 133 Gm.). It had the customary lobular shape, and the capsule showed a striking dark green color. In consistency the organ was firm but not nodular. The gallbladder was distended with a dark mucoid bile, which flowed easily from the duodenal ampulla when pressure was made on the gallbladder. Grossly the brain exhibited no kernicterus, and the microscopic sections showed no significant changes.

Microscopic Appearance of Liver.—There were small, scattered erythropoietic foci, consisting primarily of normoblasts and normocytes. Occasionally, younger cells of the erythrocytic series were found singly in the sinusoids. The lobular arrangements were more strikingly distorted than those in the previous groups. Whole lobules were shrunken with approximation of adjacent portal areas; the

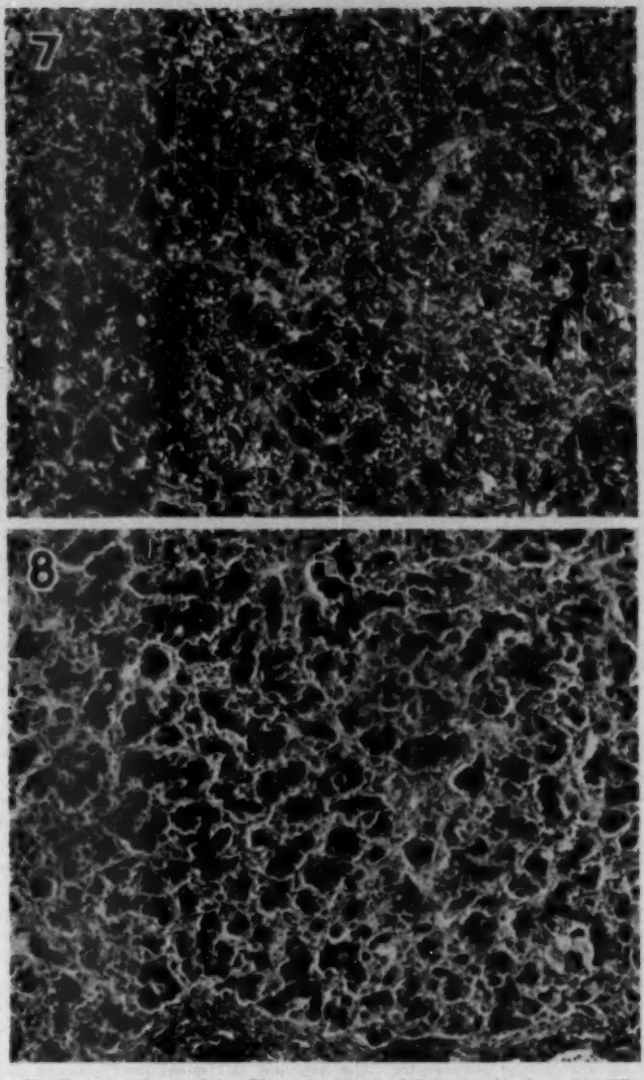


Fig. 7 (group 2, case 14).—Photomicrograph of liver showing irregular diffuse lobular fibrosis. The liver cell cords have been disrupted. (Mallory's aniline blue; × 150.)

Fig. 8 (group 3, case 15).—Photomicrograph showing cirrhosis of the liver. There is no remnant of liver cell column structure, and distortion of the lobular pattern is obvious. Necrosis and regeneration of liver cells are present. There is distinct bile stasis in the canaliculi. (Mallory's phosphotungstic acid–hematoxylin; × 150.)

latter were increased in prominence and frequently formed a fibrous juncture with adjacent portal triads. There was a diffuse increase of fibrous tissue within the distorted lobule, a feature noted previously in the other groups. Fibrous tissue surrounded and separated small groups of cell. The reticular framework was collapsed, and there was proliferation of fibrous tissue. The parenchymal cells were involved by widespread zones of necrosis with loss of tinctorial properties. They showed diffuse distribution of bile pigment and loss of nuclear chromatin. Frequently polymorphonuclear cells were found among the necrotic parenchymal cells. Bile stasis was prominent in the parenchymal cells and in the canaliculi, which were greatly distended and had distorted the surrounding liver cell column. The bile ducts were free of bile. Liver cell regeneration was active in the midlobular areas, with formation of multinuclear cells, and in the peripheral zones, where bile duct proliferation was active. Iron deposition was pronounced in the parenchymal and the Kupffer cells. The latter were more heavily impregnated than the parenchymal cells, and the macrophages in the meshes of the portal connective tissue were the most severely involved. The Kupffer cells also contained a large amount of bile pigment.

Case 16.—The mother of this infant had given birth to four children. Her first pregnancy resulted in the delivery of normal twins. The second pregnancy and delivery were uneventful. It is interesting to note that in 1945, six years after the birth of the infant under discussion, the mother, in the twenty-eighth week of her fourth pregnancy, again gave birth to twins. At that time it was discovered that her blood was free of any Rh factor and had a high anti-Rh agglutinin titer. The husband's blood contained an Rh factor. One twin was stillborn, but the other lived, although repeated transfusions were required. The placenta of this pregnancy showed changes typical of erythroblastosis.

The patient, a product of the third pregnancy, weighed 9 pounds (4,082 Gm.) at birth and was slightly jaundiced. Bile was found in the stools, and there was oozing of blood from the umbilicus. On the fifth postnatal day the jaundice began to increase and was quite distinct when the patient was admitted to the hospital. At that time the infant was drowsy but cried vigorously and had a temperature of 101 F. The spleen was palpable, and the liver was felt 3 fingerbreadths below the costal margin. No neurologic abnormalities were evident. Examination of the blood revealed 2,000,000 erythrocytes per cubic millimeter of blood, a hemoglobin level 46 per cent of normal and 10,000 leukocytes per cubic millimeter of blood, with 10 per cent nucleated cells of the erythrocytic series. In spite of repeated transfusions, the red blood cell count slowly decreased. The patient gained weight, but still the jaundice increased. One week after admission, the number of nucleated cells of the erythrocytic series had fallen to 200 per cubic millimeter of blood. The stools remained bile stained. During the second week more transfusions were given, but vitamin K deficiency appeared, and bile was found in the urine. In the latter part of the third week the jaundice deepened and generalized edema appeared. The infant died on the twenty-first day of life. Antemortem blood cultures were negative.

At postmortem examination severe jaundice was observed, with ecchymoses over the thighs and the abdomen. The lungs showed diffuse interstitial pneumonia and chronic pneumonitis. The liver and the spleen were enlarged and weighed together 189 Gm. (normal weight, 139 Gm.). On the cut surface the splenic pulp everted from the capsule, but the malpighian bodies were not perceptible. In consistency the liver (fig. 9) was firm; a thin capsule covered a dark green nodular surface tinged with brown. On section the parenchyma cut with

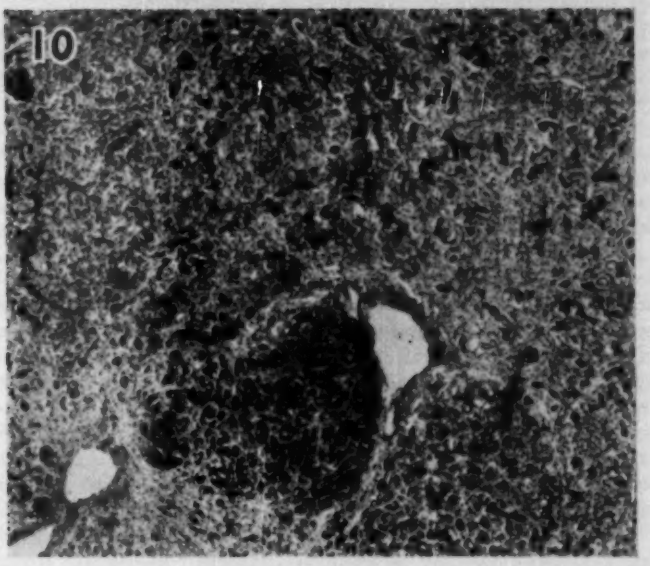


Fig. 9 (group 3, case 16).—Gross photograph of the liver. The under surface shows occasional small, irregular nodules with depressed liver substance surrounding them.

Fig. 10 (group 3, case 16).—Photomicrograph showing cirrhosis of the liver. Severe, irregular necrosis, fibrosis and parenchymal regeneration are present. Two nearly intact lobules are seen in the top center. There is notable proliferation of the bile ducts. (Mallory's hemofuchsin; × 140.)

increased resistance. The cut surfaces were irregularly lobulated and everted above the capsule. No lobular pattern was discernible. The gallbladder contained bile, and the extrahepatic bile ducts were patent. No other significant changes were found. Nuclear damage in the brain was not evident.

Microscopic Appearance of Liver.—The liver (fig. 10) contained foci of erythropoietic cells in the well preserved lobules and also in those less intact. The foci were composed predominantly of normocytes. The total lobular arrangements were distorted, but the portal triads outlining the lobules could be distinguished and were greatly increased in width. An aniline blue stain showed a large deposition of collagen in the triads. In some cases fibrous tissue almost replaced the lobular areas, isolating small groups or single parenchymal cells; in other cases fibrosis similar to that in group 2 broke up the liver cord arrangements. Parenchymal cells undergoing necrosis with vacuolation were scattered in the connective tissue which had replaced the lobules. There were granular changes in the cytoplasm, loss of nuclei and diffuse bile droplets in these cells. Parenchymal regeneration was evident from the large number of multinuclear cells present in the lobules and the pronounced pseudo bile duct proliferation at the edges of the lobules. There was no regeneration by new lobule formation. Iron stains revealed a large deposition of iron in the Kupffer cells and in the macrophages in the portal areas. The parenchymal cells, including those undergoing necrosis, as in the multinucleated regenerating cells, contained little iron. Polymorphonuclear cells were found in some of the necrotic parenchyma. The bile ducts contained no bile. Only a few bile canaliculi could be recognized in the greatly distorted liver patterns.

COMMENT

Out of a total number of 141 infants whose deaths were due to erythroblastosis only 11 per cent showed severe anatomic damage. The incidence of the histologic changes seemed independent of the parity of the mother, the severity of the disease, the postnatal survival of the infant and the related pathologic conditions.

It is certain that the processes of necrosis, cell regeneration and fibrosis occur in both antenatal and postnatal life. Prior to birth, because of the manner in which fetal blood is distributed from the umbilical vein, a portion of any maternal antibodies that may have traversed the placental barrier is received, undiluted, by the liver. The brain receives the next most pure portion of placental blood from the crossing of the blood streams in the heart. At the time of delivery the passage of the larger molecules and complete antibodies may take place more easily, owing to the high intrauterine pressure during contraction. The postnatal continuation of hepatic damage is less easily explained. However, it is definitely known that a liver containing a large number of erythropoietic elements has a high rate of anaerobic glycolysis [11] and oxygen intake. Not only does the liver support this increased metabolic demand, but it has the added burden of anemic blood, with poor carrying power for oxygen. These may be important factors in those cases in which damage continues. Immature cells in the peripheral blood stream have a short

11. Norris, J. L.; Blanchard, J., and Povolny, C.: Arch. Path. **34**:208, 1942.

existence [12] and perpetuate anemia. Yet the appearance of the liver is not that characteristically associated with anoxemia or with anoxemia in combination with toxemia.

Hoffmann and Hausmann [13] have suggested that hepatic damage is essential to the development of kernicterus. Consequently it would be reasonable to expect that a large number of patients in this series would have severe damage of the brain. Although 8 of the 10 infants in group 1 exhibited clinical signs of kernicterus, only 5 revealed anatomic evidence. In group 2 the one infant who lived more than a day had kernicterus; in the others, grossly, there was no staining of the nuclei.

The development and the pattern of diffuse lobular fibrosis can be traced in this study of the hepatic damage occurring in erythroblastotic infants. In the final stages, when fibrosis has become definite cirrhosis, regeneration of the liver takes place in a multicentrific manner and not in a manner to form new lobules. Thus, more of the existing portal triad relationships are preserved, although large areas of the parenchyma may be destroyed. This type of regeneration tends to keep the fibrosis more evenly and regularly distributed without compression. It may be merely an expression of the greater regenerative powers of the infantile liver.

The manner in which the iron pigment is distributed in the Kupffer and parenchymal cells is an interesting and unexplained feature. It seems probable that the iron which appears within the parenchyma does not imply malfunction or damage of the polygonal cells as it may in other situations.[13] Here the iron content is much decreased in the cells most damaged. The amount of iron gradually diminishes two to three weeks after birth.

The iron content of the parenchyma does not parallel the degree of bile stasis found in the bile canaliculi or the quantity of bile in the parenchymal cells, since a large amount of iron pigment may be present in the polygonal cells with little bile stasis. The prominence of bile in the parenchymal cells often denotes degenerative change.

SUMMARY

A study of the development of cirrhosis of the liver in erythroblastosis fetalis is presented. The typical pathologic changes of the liver of the erythroblastotic infant are reported. Possible etiologic factors in the production of infantile cirrhosis are discussed.

At postmortem examinations of erythroblastotic infants, various degrees of liver damage were observed. Of a total number of 141 infants examined in the present series, 10 had necrosis of the liver, 4 necrosis and fibrosis and 2 had actual cirrhosis.

12. Mollison, P. L.: Arch. Dis. Childhood **18**:161, 1943.
13. Gillman, J., and Gillman, T.: Arch. Path. **40**:239, 1945.

In the first group, comprising 10 patients, the necrosis was midzonal and easily recognized; in the second group, 4 patients, the reticulum content was increased, and fibrosis with areas of necrosis and regeneration of polygonal cells was found; in the third group there were active cirrhosis, necrosis, fibrosis and parenchymal regeneration.

The data presented support the conclusion that erythroblastosis fetalis of the Rh incompatibility type is a cause of infantile cirrhosis in a small percentage of cases.

CALCIUM DEPOSITS IN THE RENAL PAPILLAE

Their Frequency and the Various Pathologic States with Which They Were Associated in a Series of Necropsies in Jerusalem

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A SEARCH was made for renal papillar calcification in a series of necropsies performed at the Hadassah University Hospital in Jerusalem between Jan. 1, 1946 and May 30, 1948. The investigation was suggested by a number of reports appearing during the last decade,[1] in which a decisive role in the genesis of renal calculi was postulated for calcification of the renal papillae. In the opinion of Randall [1a] and his followers it is the frequency of papillary calcification which predisposes a population to nephrolithiasis, while the other factors assumed to be involved in the causation of the disease, such as electrolytic imbalance of the urine or local inflammation, were considered by them to be of secondary importance.

Palestine was considered a "stone country" by some authors [2] and was designated as such by Higgins [3] in a world map of "stone areas." However, I am not aware of any investigations concerning the climatologic, epidemiologic, social, racial and other factors of possible importance in the "geographical pathology" of the disease in this country.

In almost every discussion of renal calcification attention has been directed toward the possible pathogenic role of certain diseases. However, the relevant evidence available in the literature is confusing. This may be due in part to the difficulty of deciding at necropsy whether calcium salts were deposited during the principal disease preceding death or in the course of an intercurrent disease some time previously. In the present investigation an attempt has been made to clarify this

From the Department of Pathology of the Hebrew University Medical School and the Hadassah University Hospital.

1. (*a*) Randall, A.: Internat. Abstr. Surg., in Surg., Gynec. & Obst. **71**:209, 1940. (*b*) Rosenow, E. C. Jr.: J. Urol. **44**:19, 1940. (*c*) Anderson, W. A. D.: Ibid. **44**:29, 1940. (*d*) Vermooten, V.: Ibid. **46**:193, 1941. (*e*) Posey, L. C.: Ibid. **48**:300, 1942.

2. Garry, G., and Druckmann, A.: Ztschr. f. Urol. **24**:200, 1930. Gruber, G. B.: Die Harnsteine, in Henke, F., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1934, vol. 6, pt. 2.

3. Higgins, C. C.: Renal Lithiasis, Springfield, Ill., Charles C Thomas, Publisher, 1943.

point by the use of statistical methods testing the closeness of the association that has been noted between papillary calcification and various diseases.

MATERIAL AND METHODS

Calcium deposits were sought in the renal papillae in 250 consecutive necropsies of persons more than 20 years old, excluding those with severe lesions of the urinary tract which had caused destruction of most of the papillae. All the calices were dissected, exposing the papillae, which were examined macroscopically. Only in doubtful cases was the diagnosis controlled by histologic examination.

TABLE 1.—*The Frequency of Calcium Deposits in Renal Papillae as Reported from Different Geographic Regions and in Different Racial Groups.*

Name of Author	Country	Number of Autopsies	Age Limits	Calcification of Papillae: No.	Calcification of Papillae: Per Cent	Comment
Lubarsch [4a] (1925)	Germany	304	?	50	16.4	
Randall [7a] (1940)	Pennsylvania, U.S.A.	1,154	All ages	227	19.6	
E. C. Rosenow Jr.[7b] (1940)	Minnesota, U.S.A.	220	?	88	22.2	
Anderson [1c] (1940)	Tennessee, U.S.A.	1,300	All ages	160	12	Mixed white and colored population
		960	Adults	149	15.5	
		340	Infants and children	31	9.4	
Vermooten [14] (1941)	South Africa	1,060	All ages	88	8.3	7.2% healthy persons 17% sick persons
		65.9%	Bantus		4.3	
		26.4%	Caucasian		17.2	
		7.7%	Indian and mixed		7.3	
Kjølhede and Lassen [6] (1942)	Denmark	135	16 years and above	86	63.7	
Posey [5a] (1942)	Alabama, U.S.A.	340	Including infants	65	19.4	Mixed white and colored population
		294	21 years and above	68	23.8	
Ungar (1943)	Israel	250	20 years and above	47	18.8	Including cases of violent death of healthy persons
		[215	20 years and above	43	20.0]	Persons dying of disease

As in all previous studies,[4] papillar calcification was found to fall into two types of different macroscopic and histologic aspects. In one group of cases whitish gray plaques were noted, sharply defined, on the surface of the papillae. Histologic examination of these revealed that precipitation had occurred in the intertubular connective tissue, beginning with calcification of the basal membrane of the collecting tubule (fig. 1*A*). This form of calcification was considered to be due to degeneration of the intertubular connective tissue, and was designated by Randall and later authors as "type I lesion."

In other cases the lesion known to the older authors as "calcium infarction," referred to by Randall as "type II lesion," was observed. Histologically, it is evidenced by calcium salts forming cylindroids or accumulations of microliths within the tubules. Here, as reported by earlier authors, the two types of calcification occurred simultaneously in many instances (fig. 1*B*).

4. (*a*) Lubarsch, O.: Die Kalkablagerungen in der Niere, in Henke, F., and Lubarsch, O.: Handbuch der speziellen Pathologie und Histologie, Berlin, Julius Springer, 1925, vol. 4, pt. 1. (*b*) Goldstein, A. E., and Abeshouse, B. S.: Radiology **30**:544, 1938. Randall.[7a]

With the method of examination described here, lesions of the intertubular degenerative type ran little risk of being incorrectly diagnosed; but obviously, intratubular calcification was recorded only when the calculi had attained a

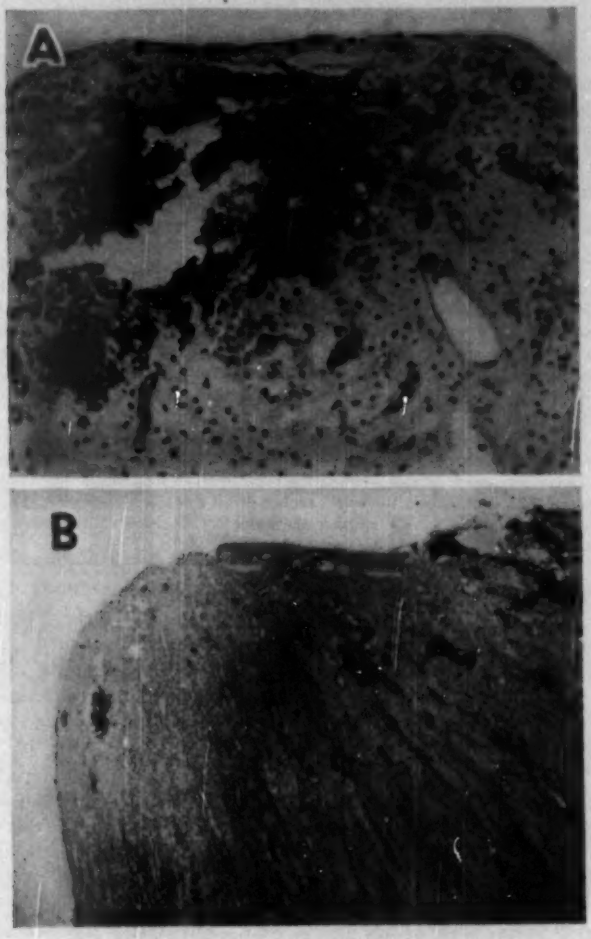

Fig. 1.—*A*, intertubular calcium deposits ("degenerative type") in a renal papilla; × 180. *B*, calcium casts filling the lumens of collecting tubules. Near the upper right border there are also intertubular calcium deposits reaching the surface of the papilla. × 25.

minimal size visible to the naked eye. For the purpose of statistical elaboration the calcifications were recorded in two classes—"calcification that might be either or both types" and "calcification of degenerative type"; I refrained from treating calcification of type II as a separate group because of the greater prominence of the subjective factor in diagnosis, as mentioned before.

CLASSIFICATION OF MATERIAL

The series of 250 necropsies was divided into the following groups with respect to the diagnostic findings: (1) diseases of bone, (2) diseases of the alimentary organs, (3) diseases of the cardiovascular system, (4) chronic diseases other than those in groups 1 to 3, (5) acute infectious diseases and (6) violent death.

The data were regrouped for testing the claims that prolonged resting in bed, infection and stasis of the urinary tract or aging may contribute to the precipitation of calcium salts.

STATISTICAL PROCEDURE

Two methods were employed for testing whether an association exists between papillar calcification and certain pathologic states; or, in other words, whether the incidence of calcium deposits in a given group of cases of disease differs from the normal. 1. A group to serve as a "normal standard" was selected by choosing from among the subjects coming to necropsy those free of any of the diseases suspected of being associated with calcification. The incidence of papillar calcification in each group of cases of disease was then compared with that in the standard group.

2. The second approach to the problem was a purely mathematical one which avoids dealing with any presumptive "normalcy." The χ^2 fourfold contingency table was used, which reveals whether the ratio between the number of cases with or without calcification and the total number of all cases is significantly different for various disease groups or whether the difference is entirely within the limits of chance variation.

INCIDENCE OF CALCIFICATION OF THE RENAL PAPILLAE

Calcification was found in the renal papillae in 47 of the 250 cases examined, i. e., in 18.8 per cent of the cases. In 23 of these (9.2 per cent) degenerative calcification (type 1) had occurred near the tips of the papillae. In 7 instances type 1 calcification was accompanied by intratubular calcification (type 2). In the remaining 24 cases type 2 deposits alone were observed.

"Normal Standard" Group of Cases.—The series of 250 necropsies included 35 cases of violent death, with no important lesions. Among them were 2 in which papillar calcification was observed: In one this was of the degenerative type, in a man in the third decade of life; in the other it was of the excretory type, in a man in the seventh decade. A great majority of those cases of violent death (29) concerned persons younger than 49 years, while all age groups were about equally represented in the remainder of the sample.

There were 25 cases of acute diseases (lobar pneumonia, typhoid, acute peritonitis, acute yellow atrophy of the liver and postoperative pulmonary embolism) in which death occurred within two weeks after

the onset of the disease. Seventeen of the patients were between the ages of 20 and 49 years. Tubular calcification had occurred in 5 cases of this group and papillar calcification of the degenerative type in only 1. The group contained no case of a disease which was suspected to be associated with calcification. It was therefore combined with the cases of violent death in the proposed standard group, which thus contained 60 cases in all.

Hence, the standard group included 8 cases in which calcification had occurred (13 per cent), in 2 of which (3 per cent) it was of the degenerative type.

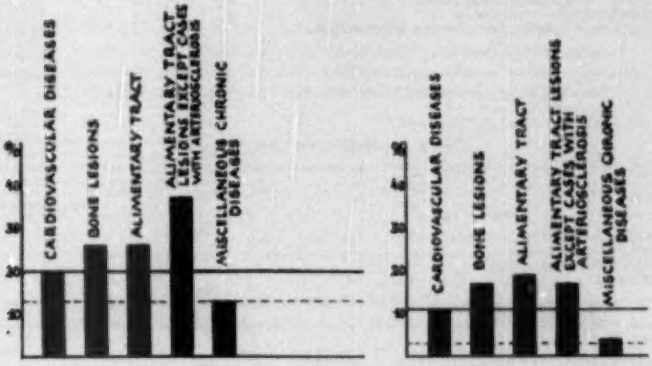

Fig. 2.—Distribution of calcium deposits among various diseases. The chart on the left gives the percentage of cases in which calcification, regardless of type, was observed; that on the right, the percentage of cases in which calcification of the degenerative type was identified. The broken horizontal line shows the percentage of cases in which calcification occurred in the "normal standard" group, and the unbroken horizontal line above it shows the percentage in the pathologic group.

Groups of Cases of Chronic Diseases Involving the Cardiovascular System, Bone, the Alimentary System and the Urinary Tract, and Other Chronic Conditions.—The findings are summarized in table 2 to 5 and are graphically presented in figure 2. The latter shows the two different percentage levels of calcification in cases of the standard group (13 per cent for calcification that might be of either or both types and 3 per cent for calcification of the degenerative type) and the corresponding levels of calcification in all "pathologic" cases (21 and 11 per cent, respectively). It is further seen that the frequency of calcification in chronic diseases is practically in the same range as that in the standard group. This group was, however, not included in the standard group, since it included the additional factor of recumbency.

Cardiovascular Disease: The group of cases of cardiovascular disease presented the specified calcifications in the same percentages as all the pathologic cases, taken together, 20 per cent and 11 per cent, respectively. Table 2 shows that in this group the greatest number of calcifications was found among cases of severe generalized arteriosclerosis.

TABLE 2.—*Cardiovascular Diseases*

Type of Lesion	Cases	"Calcification of Both Types"*	Degenerative Calcification
Severe general arteriosclerosis	38	8	3
Chronic hypertensive renal disease	10	2	1
Arteriosclerotic heart disease	14	3	2
Valvular, rheumatic and syphilitic heart disease	22	2	2
Total number of cases	74	15 (20%)	8 (11%)

* This classification is explained in the text under "Materials and Methods."

TABLE 3.—*Destructive Bone Lesions*

Type of Lesion	Cases	"Calcification of Both Types"	Degenerative Calcification
Inflammatory and metabolic lesions	6	2	2
Secondary carcinoma	22	6	4
Lymphosarcoma group	6	..	..
Myeloma	1	1	..
Total number of cases	35	9 (26%)	6 (17%)

TABLE 4.—*Diseases of Alimentary Organs*

Type of Lesion	Cases	"Calcification of Both Types"	Degenerative Calcification
Diseases of the gastrointestinal tract	26	5	3
Diseases of liver and pancreas	17	6	3
Total number of cases	43	11 (26%)	8 (19%)

Lesions of Bone; Diseases of the Alimentary Tract: The percentages of the specified calcifications in patients with lesions of bones (table 3: 26 per cent and 17 per cent) and in those with diseases of the alimentary tract (table 4: 26 per cent and 19 per cent) are considerably above the average.

Lesions of the Urinary Tract: Evidence of stasis in, or infection of, the urinary tract or a combination of both was found at necropsy in 35 cases. The lesions were associated with pelvic neoplasms which were compressing the ureters, with prostatic hypertrophy, with neurologic diseases, with general infections and with chronic pyelitis, or were due to unknown causes. The findings are summarized in table 6.

No association may be assumed to exist between papillar calcification and the discussed lesions of the urinary tract, the more so because in several cases of this group calcification may have been due to other, extrarenal factors. Among 15 cases of urinary stasis without infection the only case in which calcification of the degenerative type occurred was that in which tuberculosis of the spine was present, and the one case in which the excretory type was noted was a case in which chronic myeloid leukemia was diagnosed, with small uric acid calculi being excreted for some time.

Prolonged Recumbency: In all 250 cases, data concerning the duration of uninterrupted resting in bed were collected from the clinical

TABLE 5.—*Miscellaneous Chronic Diseases and Cancers (Except Those Included in One of the Disease Groups Mentioned in Other Tables)*

Type of Lesion	Cases	"Calcification of Both Types"	Degenerative Calcification
Miscellaneous chronic diseases (central nervous system, dermatoses, prostatic hypertrophy)	33	4	2
Cancers of hemopoietic system (except cases in which there were metastases in bone)	13	2	..
Other tumors (except of alimentary organs and with metastases in bone)	15	1	..
Total number of cases	61	7 (14%)	4 (4%)

TABLE 6.—*Pathologic Conditions of the Urinary Tract*

	Cases	"Calcifications of Both Types"	Degenerative Calcification
Stasis, no infection	15	2	1
Stasis and infection	12	1	..
Infection alone	8	..	..
Total number of cases	35	4 (11%)	1 (3%)

histories. There were 53 cases of more than four weeks and 35 cases of more than eight weeks of recumbency. These series included 11 and 7 respectively in which calcification occurred. The percentage of calcification, therefore, does not exceed the average of the entire series.

Sex Groups (table 7).—The 250 subjects of necropsy included 145 men, 34 of whom had papillar calcification (23 per cent) and 105 women, 13 of whom had papillar calcification (12 per cent). It appears, therefore, that papillar calcification is approximately twice as frequent among men as among women. The disposition of men to have it is more pronounced if cases of the standard group are excluded; the proportion of calcifications in males and females then increases, respectively, to 23 per cent (34 cases) and 12 per cent (13 cases).

The proportion remains practically the same with regard to calcifications of the degenerative type, with 11 per cent (16 cases) for men and 7 per cent (7 cases) for women.

Age Groups.—Of the 250 subjects of necropsy, 138 were between the ages of 20 and 49 years, 21 of whom (15 per cent) had papillar calcification (all types). The remaining 112 were more than 50 years of age, 26 of whom (23 per cent) had calcification. With regard to degenerative lesions the numbers were, respectively, 11 (8 per cent) and 12 (11 per cent).

The possible influence of aging on the precipitation of calcium salts could best be tested, theoretically speaking, by comparing older and younger age subgroups of the "normal" group. However, the "normal" group contained too small a number of subjects above the age of 50 to permit this procedure. Another possible method, that of testing the

TABLE 7.—*Summary of Associations of Papillar Calcification*

		"Calcification, Both Types"				Calcification, Degenerative Type			
	Cases	No.	%	χ^2	Association?	No.	%	χ^2	Association?
Total series	250	47	19	....	.....	23	9	....	.....
Standard group	60	8	13	1.56	Doubtful	2	3	....	.....
Disease group	190	39	21			22	11	....	.....
Association with disease:									
Cardiovascular disease	74	15	20	0.11	None	8	11	0.10	None
Lesions of bone	35	9	26	1.18	Doubtful	6	17	2.30	Doubtful
Lesions of alimentary organs	43	11	26	1.44	Doubtful	8	19	4.39	Present (P>0.05)
Various chronic diseases	51	7	14	0.37	None	2	4	2.35	Doubtful
Association with age:									
20 to 49 years	98*	16	17	1.97	Doubtful	10	11	0.12	None
50 and above	90*	23	24			11	11		
Association with sex:									
Male	145	34	23	4.91	Present (P>0.05)	16	11	1.02	Doubtful
Female	105	13	12			7	7		

* The number represents the total number of cases less the standard group.

age factor as a second variable in each disease group, taken separately, also proved impracticable in view of the smallness of the samples. In order to obtain at least a preliminary view of the problem we compared both age groups within the entire sample of persons with pathologic conditions (table 7).

The comparison showed no significant difference in the frequency of calcification of the degenerative type. With regard to calcification of both types, the percentage is higher in the older group than in the younger group (24, compared with 17, per cent).

TEST OF SIGNIFICANCE

The statistical data presented in the foregoing sections suggest that papillar calcification is associated with diseases as a group, and particularly with diseases of bones and of the alimentary organs. They

further indicate a frank tendency of papillar calcification to affect males. An association of advancing age and all types of calcification also seems likely.

The significance of these data was tested by the χ^2 method. χ^2 values of 3.8 (signifying that $P = 0.05$) or more are generally considered to indicate practical certainty, while somewhat lower values are considered doubtful unless larger samples are studied. When χ^2 is less than 0.5, however, even enlarging the sample is unlikely to increase significance.

χ^2 tests were performed with the series of 250 cases and with separate parts of it. In comparing the prevalence of calcification in different diseases, the control sample consisted of the sum of the numbers of cases in the different groups; there were 50 cases in which calcification was observed among 263 cases examined. We arrived at this sum because 13 cases, including 3 in which calcification was present, occurred in two different groups of diseases; 9 were cases of arteriosclerosis and 4 were cases of neoplasms of the alimentary tract with concurrent metastatic destruction of bones.

With these reservations in mind, the results may be interpreted as follows (table 7):

(a) There is a direct association between calcification of the degenerative type (Randall's type I) and diseases of the alimentary tract.

(b) There is a close association between calcification, regardless of type, and the male sex, but no significant difference was found between the sexes for calcification of the degenerative type.

(c) No significant association is found with any of the other conditions investigated.

COMMENT

General Incidence of Papillar Calcification.—The findings may be compared with the results of previous investigations in which the criteria for diagnosis were similar to ours.[1] In such reports (table 1) papillar calcifications were observed to occur in 15 to 22 per cent of adults dying of diseases. If statistical variations depending on the size of the sample are taken into account,[16] our observations fall into the same range.

It may be concluded, therefore, that the frequency distribution of calcification does not vary with the country, nor with the color of the population. A single exception is contained in a report of a significantly lower incidence of calcification in the Bantu population as compared with the white population of South Africa.[14] However, it seems premature to conclude that racial dispositions vary, since most of Vermooten's Bantu cases were taken from medicolegal necropsies of healthy persons of a lower average age than the persons in other authors' series. Fre-

quency of calcification exceeding the percentages cited in the foregoing paragraph was reported only by Kjølhede and Lassen,[5] who observed 86 (i. e., 63.7 per cent) cases in which calcification of the papilla had occurred, among 135 necropsies. However, the macroscopic description of "calcified plaques" given by these authors appears to be identical with that of "lipoid infarct" of the renal papillae.[4a] In our experience this finding sometimes appeared on histologic examination of doubtful cases (fig. 3), which may have been mistaken for calcification.

Association of Calcification of Renal Papillae and Various Pathologic States.—The Literature: Deposits of calcium salts in the renal papillae in relation to disease have been studied from the time of Henle's description of "calcium infarction of the kidneys," about one hundred years

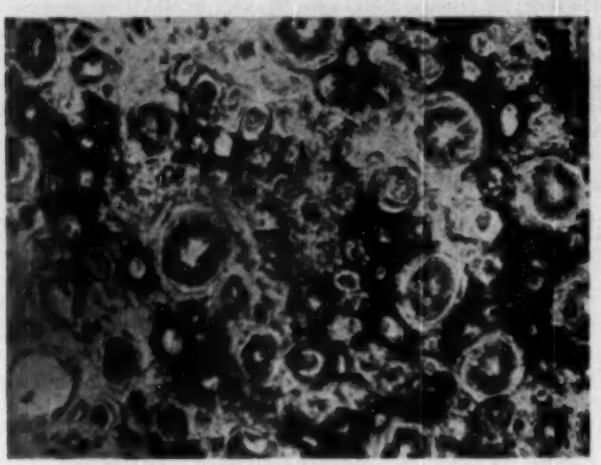

Fig. 3.—Cross section of a renal papilla showing intertubular deposits of fatty substances. Stained with sudan III; × 190.

ago. The literature was surveyed by Goldstein and Abeshouse,[4b] who listed reports of renal calcification accompanying various infectious and neoplastic processes, diseases of the skeleton and alimentary organs and especially cirrhosis of the liver. The possibility that calcification is related to renal dysfunction in intestinal obstruction was discussed by Martz.[6] More recently, Flocks [7] mentioned the calcium precipitated in the tubules in cases of hypercalcinuria owing to a number of conditions accompanied by mobilization of skeletal calcium, such as prolonged

5. Kjølhede, K. T., and Lassen, H. K.: J. Urol. **47**:45, 1942.
6. Martz, H.: Arch. Int. Med. **65**:375, 1940
7. Flocks, R. H.: J. A. M. A. **130**:913, 1946.

immobilization of large bones following trauma or osteomyelitis, or prolonged recumbency due to chronic visceral disease.

Pathologic, statistical and experimental studies [8] have shown that renal calcification may be caused by hyperparathyroidism, but with regard to other diseases the statistical evidence has so far remained inconclusive. In studies based on large series of necropsies some authors have suggested the possibility that renal calcification is associated with generalized arteriosclerosis [9]; others have stressed the primary importance of vascular lesions of the kidneys,[10] or have explained the calcium deposits as a result of age.[10] According to Randall,[10a] calcification of the papillae may be a result of tubular damage caused by the bacterial toxins excreted during intercurrent general infections. Rosenow [10b] is inclined to deny that there is a causal relation between renal calcification and any special disease.

The Presented Material: No contribution can be made by statistics alone to the resolution of questions of etiologic relationships. Therefore, results pointing to significant associations between renal calcification and normal physiologic or pathologic conditions can serve only to support, or in certain instances to raise doubts with regard to, conclusions drawn from investigations in isolated small series of cases.

The percentage of calcification in 190 cases of death of disease appears considerably higher than that in the "standard group" (fig. 2). Nevertheless, by means of the χ^2 test, this difference could not be proved to be beyond the range of chance variations.

The absence of any significant association of calcification and destructive lesions of bones in our series is opposed to the views of the older authors who considered these lesions to be the most frequent source of the renal lesions.[4a] Nevertheless, the χ^2 value is high enough to suggest that the data may still become significant if the series is enlarged. However, we avoided extending the period of observation beyond the spring months of 1948 because of conditions which were likely to influence the results of a study of this kind, such as the abnormal nutrition during the siege of Jerusalem and recent immigration.

No association was revealed for cardiovascular diseases, prolonged recumbency or infection and stasis of the urinary tract. The negative results in the group of cases of prolonged recumbency and in that of cases of infection and stasis of the urinary tract are noteworthy in view of the numerous reports claiming a frequent occurrence of renal

8. Anderson, W. A. D.: Endocrinology **24**:372, 1939. Albright, F.; Baird, P. C.; Cope, O., and Bloombery, E.: Am. J. M. Sc. **187**:49, 1934. Castleman, B., and Mallory, T. B.: Am. J. Path. **11**:1, 1935.

9. Anderson,[1c] Vermooten,[1d] Kjølhede and Lassen.[5]

10. Anderson, L., and MacDonald, J. R.: Surg., Gynec. & Obst. **28**:275, 1946. Schmidt, M. B.: Die Verkalkungen, in von Albrecht, E.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1921, vol. 3, pt. 2.

and ureteral calculi in these conditions. It appears that when such observations can be proved to be statistically correct, the origin of the calculi in the latter group is not likely to be correlated with calcification of the papillae.

The observation that "calcification of both types," but not "calcification of the degenerative type," was associated with the male sex was found to be significant. This observation agrees with earlier reports stating that papillar calcification is two to three times more prevalent in males than in females.[1b, c, d]

Calcification of the degenerative type was found to be significantly associated with cases of disease of the alimentary tract. It may be concluded, therefore, that the statistical observations support the views on the causal relationships existing between renal calcification and gastrointestinal diseases [11] and chronic hepatic disease [12] which were obtained from the study of isolated cases.

SUMMARY

The frequency of calcium deposits in renal papillae was studied in 250 consecutive necropsies of persons above the age of 20 years in the Jewish part of Jerusalem.

The results of the inquiry are comparable to those reported from other countries with regard to frequency and the influence of age and race.

The degree to which calcification of the intertubular (degenerative) type and the intratubular (excretory) type was associated with various pathologic states, age and sex was investigated. The statistical values were elaborated by the χ^2 test in order to eliminate the possibility of their being due to chance variations.

No association was present for *(a)* cardiovascular diseases, *(b)* prolonged recumbency and *(c)* infection and stasis of the urinary tract.

The percentage of cases with calcium deposits in the group of destructive lesions of bones and the group of diseases of the alimentary organs was higher than the combined average of all cases of death of disease. However, the mathematical test proved the results to be statistically significant only with regard to the association between calcification of the degenerative type and disease of the alimentary organs.

Calcium deposits were observed in the renal papillae more than two and a half times more frequently in men than in women.

Help and advice respecting the treatment of statistical data were given by Uriel Foa, Ph.D.

11. Goldstein and Abeshouse.[4b] Martz.[6]

12. Goldschmidt, E.: Abstracted in Centralbl. f. allg. Path. u. path. Anat. **24**:647, 1913. Roessle, R.: Entzündungen der Leber, Berlin, Julius Springer, 1929, vol. 5, pt. 1. Askanazy, M.: Compt. rend. de la deuxième Conf. Internat. de Path. Géographique, Utrecht, 1934. Posey.[1e] Kjølhede and Lassen.[5]

EXPERIMENTAL CORONARY SCLEROSIS

III. Lymphomatosis as a Cause of Coronary Sclerosis in Chickens

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PREVIOUS PAPERS in this series have been concerned with the incidence of arteriosclerosis of the coronary arteries in adult white leghorn chickens, with the primary lesion that initiates the sclerotic process in these vessels and with the effects which various experimental procedures have on the incidence of the disease.

Coronary sclerosis is extremely common in adult fowl. When the coronary arteries are studied by means of serial sections, more than 70 per cent of 1 year old birds show some evidence of the disease.[1] Comparative studies of various types of arteries have not been attempted, but it would appear that the coronary arteries are more susceptible than are other vessels, including the aorta.

Complete serial section through early sclerotic lesions has shown that the primary change is a focus of cellular infiltration and "hydropic" degeneration of the medial coat of the artery, and that proliferation of the intima follows as a secondary manifestation.[1]

The feeding of cholesterol to chickens for prolonged periods does not change the incidence of the disease from that seen in control birds.[2] It is concluded that this procedure is not concerned etiologically in the coronary sclerosis of chickens, although it does have important secondary effects: individual plaques become infiltrated with lipoid material and increase in size. The accelerating effect of cholesterol feeding is, to a degree, reversible; withdrawal of excess cholesterol from the diet is followed by regression in the size of the plaques and by a decrease in the amount of lipoid material deposited.[3]

This study was aided by a grant from the National Research Council of Canada.

From the Department of Medical Research, University of Western Ontario, London, Canada, and the Regional Poultry Research Laboratory, United States Department of Agriculture, East Lansing, Mich.

1. Paterson, J. C.; Slinger, S. J., and Gartley, K. M.: Arch. Path. **45**:306, 1948.

2. Paterson, J. C.; Mitchell, C. M., and Wallace, A. C.: Arch. Path. **47**:335, 1949. Paterson and others.[1]

3. Paterson, J. C., and Gartley, K. M.: Unpublished observations.

Isolating chickens against infectious diseases of an acquired type has not changed the incidence of coronary sclerosis from that seen in control chickens.[2]

Because of the negative results obtained in the experiments just mentioned, we have studied, finally, the possibility that coronary sclerosis may be related to an extremely common neoplastic disease of chickens —lymphomatosis. An investigation has been carried out in which the incidence of arterial lesions in birds infected experimentally with the causative agent of lymphomatosis was compared with that in control birds. The results, reported here, suggest that the primary medial lesion under discussion may be related etiologically to this neoplastic disease.

MATERIAL AND METHOD

The experimental procedures were carried out at the Regional Poultry Research Laboratory, United States Department of Agriculture, East Lansing, Mich.

The series consisted of 58 newly hatched female single-comb white leghorn chicks from a line in which lymphomatosis had been reduced to a minimum by selective breeding and isolation over many generations. This line (line 15) has been found to be highly susceptible to experimental infection with lymphomatosis, both by natural exposure and by inoculation.[4] When birds of this line were raised in isolated pens, they had a low incidence of naturally occurring lymphomatosis.[5]

The series was divided into two groups, which were treated as follows: Group 1, the control group, consisted of 34 chicks which had been hatched and reared in isolation. At 2 days of age they were given an intratracheal instillation of pooled tracheal and nasal washings from 12 adult birds which were not suffering from clinical or gross evidence of lymphomatosis. These 12 donor birds were sisters, and some of them were direct ancestors of the recipient chicks. The donor birds were 5½ years old at the time they were killed, and they had been hatched and reared as a family unit in an isolated pen. During the five and a half year period, none of the sisters that died showed clinical or gross evidence of lymphomatosis. Thus, this group of birds constituted the nearest approach to a group freed from lymphomatosis that has been attained at the United States Regional Poultry Research Laboratory.[5a] Of the 34 recipient birds in group 1, 32 survived to the age of 339 days, when they were destroyed. The coronary arteries of 30 of these surviving birds were examined for evidence of coronary sclerosis.[6]

Group 2, the infected group, consisted of 24 chicks from the same line as group 1.[7] They were hatched in the same isolated incubator as group 1, but were hatched two weeks after the first group. They were hatched and reared in an isolated pen

4. (*a*) Waters, N. F.: Poultry Sc. **24**:226, 1945. (*b*) Waters, N. F., and Bywaters, J. H.: Ibid. **28**:254, 1949. (*c*) Burmester, B. R., and Cottral, G. E.: Cancer Research **7**:669, 1947. (*d*) Cottrall, G. E.; Burmester, B. R., and Waters, N. F.: Poultry Sc., to be published.

5. (*a*) Waters, N. F., and Prickett, C. O.: Poultry Sc. **23**:321, 1944. (*b*) Waters.[4a] (*c*) Waters and Bywaters.[4b]

6. Two specimens from group 1 and 1 specimen from group 2 were discarded because the labels were lost and positive identification could not be made.

7. The chicks in both groups were from identical families for the most part. Ten identical families were represented in both groups, the difference being that group 1 had three additional families.

adjacent to the pen housing group 1. At 2 days of age they were given an intratracheal instillation of pooled tracheal and nasal washings from 2 unrelated adult birds (both 79 days old) suffering from advanced neural lymphomatosis. Only 20 of the birds in group 2 survived to the age of 326 days, when they were destroyed and the coronary arteries of 19 of them were examined for evidence of sclerosis.[8]

The isolation technic [8] was that in routine use at the United States Regional Poultry Research Laboratory. The birds were killed by electrocution. Representative specimens from the liver, the pancreas, the spleen and a kidney of each bird were fixed in Zenker's solution, and single sections of these organs were stained with hematoxylin and eosin and examined for microscopic evidence of lymphomatosis.

The assessment of the incidence and the degree of coronary sclerosis in each bird was made by a special technic as practiced in previous experiments.[3] After the heart had been fixed in 4 per cent formaldehyde solution, four blocks were cut from the upper portions of the organ, each block being cut horizontally through the entire thickness of the organ. The first block was taken at the level of the atrioventricular groove, and the other three, consecutively, at lower levels. The blocks were embedded in paraffin, and serial sections cut at intervals of 400 microns through the entire thickness of each block. In this manner, from 20 to 30 equidistant sections, each of which usually contained about three cross sections of the main coronary arteries, were obtained from each specimen. The sections were stained routinely with hematoxylin and eosin and occasionally with Weigert's elastic tissue stain. In the actual assessment of the incidence and the degree of coronary sclerosis in each heart, every coronary artery measuring more than 250 microns in its widest diameter, more or less, was counted and examined. When intimal or medial disease was seen in an individual section of an artery, its degree was noted.

OBSERVATIONS

Two of the control birds died during the course of the experiment of causes other than lymphomatosis, but at autopsy 3 of the survivors showed gross evidence of this condition. The incidence of obvious lymphomatosis in this group was, therefore, 8.8 per cent. The infected group, on the other hand, showed a frank acceleration of the disease: 4 of the birds died of lymphomatosis during the course of the experiment, and autopsy of the survivors showed that 3 additional birds had cancerous tumors on gross examination. The total incidence of obvious lymphomatosis in the infected group was, therefore, 29 per cent.

Microscopic examination of multiple sections of the myocardium and of single sections of liver, spleen, pancreas and kidney of each surviving bird failed to show definite evidence of acceleration in the growth of the so-called normal lymphoid foci in the infected group. Foci of lymphoid cells were found in at least one organ of each bird in the two groups, but these foci were not significantly larger or more numerous in the infected birds than in the control birds.[9] Of the organs

8. Waters and Bywaters.[4b] Waters and Prickett.[5a]

9. The assessment of the size and the number of the lymphoid foci was not based on actual measurement, as practiced by Lucas and Oakberg, but was made by a simple method of grading.

studied, the myocardium seemed to be the tissue most susceptible to cellular infiltration (fig. 1).

Serial section of the coronary artery system revealed arterial lesions which were identical with those described in previous reports.[2] The medial changes were the most striking. Foci of round cell infiltration, hydropic degeneration of smooth muscle cells with pyknosis of nuclei or karyorrhexis, and disintegration of elastic tissue fibers were the prominent features (fig. 2). The adventitia and the intima adjacent to the medial lesion were usually infiltrated with round cells, and the intima, in addition, showed variable degrees of fibrous tissue proliferation with the formation of definite plaques. The morphologic aspect of the arterial

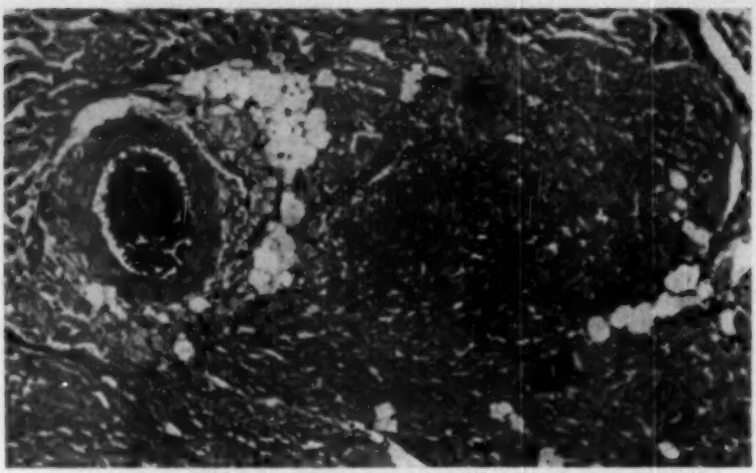

Fig. 1.—Photomicrograph of tissue from the heart of a chicken. It shows a focus of so-called normal lymphoid cells in the myocardium just to the right of a small normal artery. Hematoxylin and eosin stain; × 100.

lesions was the same in control birds and in infected birds, but in their number and size the lesions were markedly dissimilar.

The incidence of arterial lesions, determined by examining and counting every cross section of an artery over 250 microns in diameter, showed more than a sixfold increase in the infected group as compared with the control group (table 1). The actual figures were 243 lesions in 1,245 arteries in the infected birds (19.5 per cent), compared with 71 lesions in 2,365 arteries in the control birds (3 per cent).

The degree of secondary intimal proliferation at the point of medial disease was more pronounced in infected birds than in control birds

(table 2). In the control birds it was always of a slight or moderate grade; whereas, 26 per cent of the infected group showed a marked

TABLE 1.—*Incidence and Degree of Coronary Sclerosis in Control and Infected Chickens*

	Control Group			Infected Group		
Bird	Arteries Studied	Diseased Arteries	Maximum Degree of Intimal Proliferation	Arteries Studied	Diseased Arteries	Maximum Degree of Intimal Proliferation
1	71	0	0	74	42	+++
2	27	0	0	66	6	+
3	86	6	++	98	12	++
4	87	0	0	37	2	+
5	103	0	0	54	7	+
6	101	19	+	37	1	+
7	72	0	0	54	21	+++
8	104	4	+	65	46	+++
9	109	1	+	46	0	0
10	71	1	+	76	2	+
11	72	0	0	46	1	+
12	79	1	+	83	4	+
13	71	1	+	57	6	+
14	71	2	+	74	3	+
15	96	6	+	56	6	+
16	100	0	0	55	9	+++
17	63	13	++	81	47	++++
18	86	0	0	59	21	++
19	97	12	++	79	5	+
20	96	0	0	..	..	.....
21	69	1	+	..	..	.....
22	76	0	0	..	..	.....
23	90	0	0	..	..	.....
24	72	2	+	..	..	.....
25	51	4	+	..	..	.....
26	46	0	0	..	..	.....
27	103	0	0	..	..	.....
28	62	0	0	..	..	.....
29	63	0	0	..	..	.....
30	70	5	++	..	..	.....
Total	2,365	71	..	1,345	243	.....
Average	79	2 (3%)	..	66	15 (19.6%)	.....

TABLE 2.—*Degree of Coronary Sclerosis in Control and Infected Birds*

	Percentage Showing Given Degree of Sclerosis		
	Slight	Moderate	Marked
Control birds (30 in all).....	37	13	0
Infected birds (19 in all).....	58	11	26

degree of intimal thickening. Examples of slight, moderate and marked grades of intimal proliferation are illustrated in figures 2, 3 and 4 respectively.

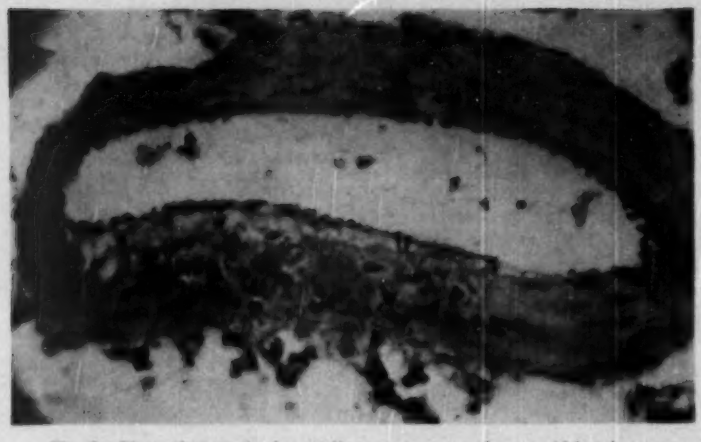

Fig. 2.—Photomicrograph of a small coronary artery from a chicken in a previous experiment. The medial coat shows hydropic degeneration and necrosis of muscle fibers. The adjacent intimal layer is thickened, and this degree of thickening is assessed as "slight." Hematoxylin and eosin stain; × 300.

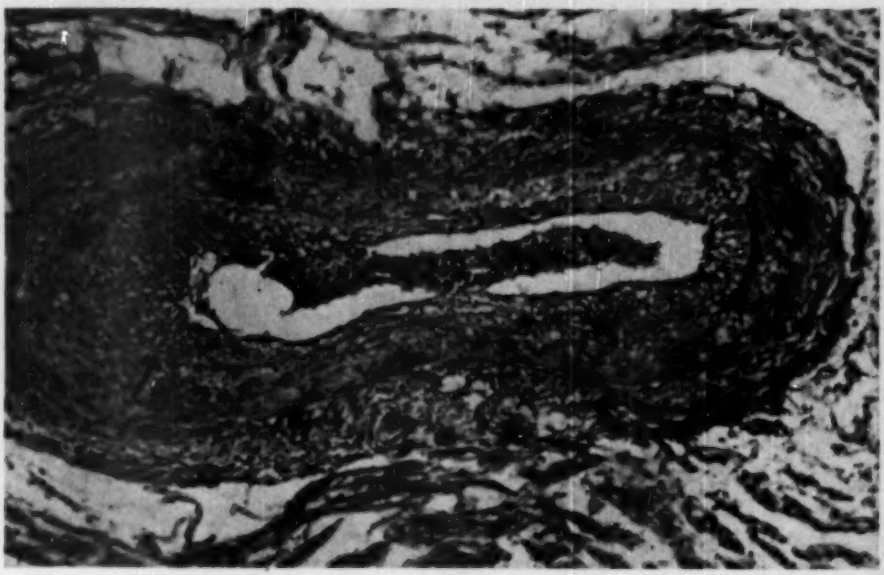

Fig. 3.—Photomicrograph of a coronary artery showing a degree of intimal proliferation assessed as "moderate." The arrow points to the internal elastic lamina. Several areas of slight cellular infiltration and hydropic degeneration of the medial coat are seen. Hematoxylin and eosin stain; × 100.

Summarizing, then, we find that the experiment in which respiratory washings from birds with neural lymphomatosis were introduced into the tracheas of chicks resulted in (1) either acceleration or transmission of the natural type of disease as determined by gross examination of various organs, (2) a sharp increase of the mortality from neural and visceral lymphomatosis and (3) a definite acceleration in the number and the size of the lesions of the coronary arteries—lesions which have been regarded heretofore as representing a "spontaneous" type of arteriosclerosis.

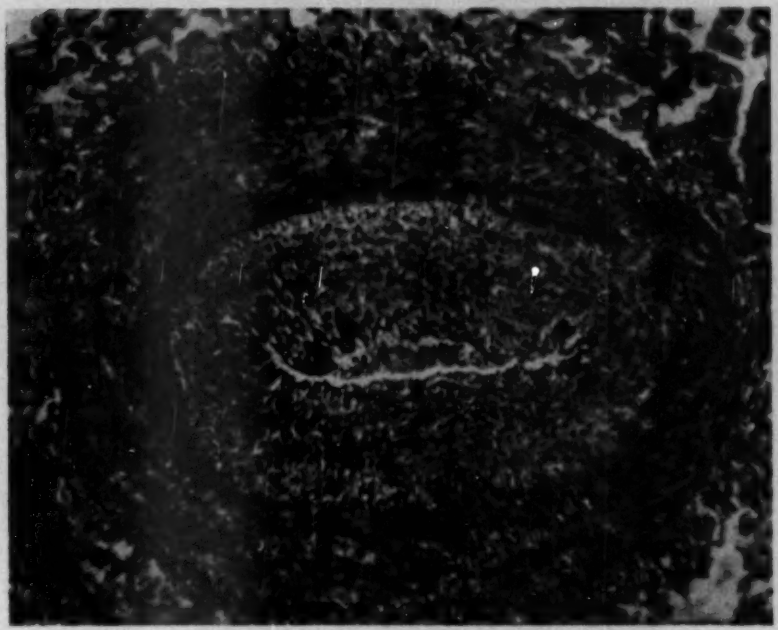

Fig. 4.—Photomicrograph of a coronary artery. The arrow points to the internal elastic lamina. The proliferation of the intima is excessive and has resulted in stenosis of the lumen. This degree of intimal proliferation is assessed as "marked." The media shows slight but definite cellular infiltration at some points. Hematoxylin and eosin stain; × 120.

COMMENT

The results of this experiment, while highly suggestive, are perhaps inconclusive, since our control birds were not entirely free from coronary sclerosis and from lymphomatosis. In our experience, involving chickens reared in different parts of Canada and in Michigan, no strain of mature birds exists that is free from arterial lesions; nor has a lympho-

matosis-free flock of chickens ever been achieved to our knowledge. Strict isolation and selective breeding will eventually yield a stock which is almost completely free from the clinical and gross pathologic manifestations of lymphomatosis, but careful examination of the viscera will reveal slight but definite stigmas in practically every bird.[9] Our experimental material was drawn from a stock of this type. The control birds and the infected birds were reared under conditions which were identical except in one respect—one group was inoculated with tracheal and nasal washings from birds which were free from obvious lymphomatosis while the other group was inoculated with washings from birds with severe lymphomatosis. A clearcut difference in the incidence and the degree of arterial lesions resulted. There also was a difference between the two groups in the incidence of gross lymphomatosis.

Previous experiments have shown that lymphomatosis can be transmitted by instilling respiratory washings from birds with neural lymphomatosis into susceptible chicks via the respiratory route.[10] Thus it appears that the respiratory washings from certain infected birds contain the agent of the disease. The respiratory washings contain factors other than the agent of lymphomatosis, namely, bacteria, mucus, desquamated cells and possibly other unknown viral agents. Therefore, it cannot be denied that factors other than the agent of lymphomatosis might have been responsible for the acceleration of the coronary lesions. Two arguments may be advanced against this suggestion: (1) The respiratory washings from noninfected donors that were instilled into our control birds should have contained the same hypothetic accelerating factors as the washings from infected donors, and (2) we have been completely unsuccessful, in the past, in influencing the incidence of arterial lesions by inoculating bacteria, by isolating against infectious disease of the ordinary, acquired variety, and by other measures.[2] The logical tentative conclusion, therefore, is that in chickens either the agent of lymphomatosis or the disease process of lymphomatosis may be associated with the production of lesions which we recognize as arteriosclerosis. The round cell infiltrations in intima, media and adventitia appear to represent foci of lymphomatous infiltration, and the secondary intimal proliferation is interpreted as a response to the presence of these cells. Figure 5 illustrates the essential cellular and, possibly, neoplastic nature of the primary medial lesions.

Supporting evidence for the belief that the arterial lesions are related to lymphomatosis has been obtained from a second experiment recently completed. Thirteen female white leghorn chickens of the same strain as those in the main experiment were exposed to contact infection with infected birds at an early age. They were killed when they were 300 days old, and the incidence and the degree of lesions of the coronary

10. Winton, B.: Ninth Annual Report of the Regional Poultry Research Laboratory, East Lansing, Mich., 1948, pp. 1-21.

arteries were assessed in the usual manner. In this group 1,033 arteries were examined and, of these, 256, or 25 per cent, were diseased; and in 10 of 13 birds the degree of secondary intimal proliferation was graded as "marked." The results of this experiment suggest that contact exposure is just as effective as intratracheal instillation in accelerating the incidence and the degree of arterial lesions of the type under discussion.

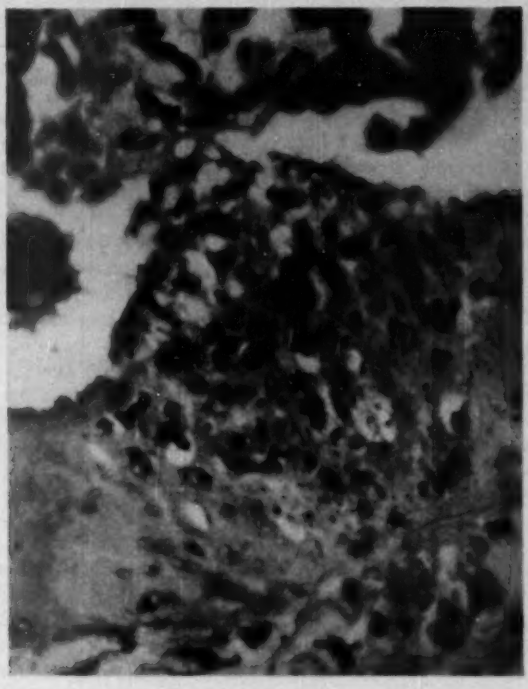

Fig. 5.—Photomicrograph of an early lesion of the wall of a coronary artery, showing the essential cellular, and perhaps neoplastic, nature of the medial lesion. The adjacent intima is hyperplastic and is infiltrated with similar cells. Hematoxylin and eosin stain; × 450.

Many workers have observed the intimate vascular association of the early lesions of lymphomatosis. These early lesions usually are adjacent to or around veins. Oftentimes, the walls of the veins are invaded by the neoplastic cells. Severe subendothelial infiltration, to a degree that may result in partial occlusion of the vein, is sometimes found.

Lucas and Oakberg [11] have recently reviewed the subject of the "so-called normal lymphoid foci" that are frequently found in the various visceral organs of chickens and other birds. They concluded that these lymphoid foci were usually associated with vessels, that most birds had such lymphoid foci in their visceral organs and that these foci were not normal structures. They further suggested that the agent of lymphomatosis may be the inciting cause of these lymphoid foci. It is of interest to note that Pappenheimer, Dunn and Cone,[12] in 1926, in their classic studies on neural lymphomatosis, described "interesting cellular infiltrations" in the media and intima of the arteries of the leg in 2 birds with gangrene of the feet, and they implied that these vascular infiltrations were lymphomatous.

Thus, the evidence suggests that the avian arterial disease may be a form of lymphomatosis instead of a nonspecific arteriosclerosis. Lymphomatosis is generally regarded as a naturally occurring cancer of birds. The causative agent is filtrable, and there is reason to believe that it may be transmitted from bird to bird by direct contact [13] and from parent to offspring by way of the egg.[14] The disease may manifest itself in several ways; neural, visceral, ocular and osteopetrotic varieties have been described, and from the evidence obtained in the present experiment there is reason to believe that arterial lesions may also exist and that they are extremely common.

Lymphomatosis is a disease peculiar to the avian family; it has never been transmitted to mammals, and there is, therefore, no reason to suspect that the causative agent bears any relationship to the etiology of human arteriosclerosis. On the other hand, while unrelated etiologically, the mechanism of development of the arterial disease in the two species may still be similar.

SUMMARY AND CONCLUSIONS

Single comb, white leghorn chickens of a line which had been rendered relatively free from lymphomatosis by isolation and selective breeding have been exposed to infection with virulent lymphomatous material, and the effect of this procedure on the number and the size of lesions of the coronary arteries has been compared with that in

11. (*a*) Lucas, A. M., and Oakberg, E. F.: Am. J. Path., to be published. (*b*) Lucas, A. M.: ibid., to be published. (*c*) Oakberg, E. F., and Lucas, A. M.: Poultry Sc. **28**:675, 1949.

12. Pappenheimer, A. M.; Dunn, L. C., and Cone, V.: A Study of Fowl Paralysis, Bulletin 143, Storrs Agricultural Experiment Station, Storrs, Conn., December 1926.

13. Burmester, B. R., and Dennington, E. M.: Cancer Research **7**:779, 1947.

14. Cottral, G. E., in the Proceedings of the Fifty-Third Annual Meeting of the United States Livestock Sanitary Association, 1949, Baltimore, Waverly Press, to be published. Waters.[4a] Waters and Bywaters.[4b] Cottral and others.[4d]

noninfected birds of the same line. A sixfold increase of the number of arterial lesions and a marked acceleration of the size of individual lesions were found in the infected birds as compared with the controls. From the evidence at hand it appears that these arterial lesions, considered in the past to be manifestations of arteriosclerosis, may be merely a part of the disease process of lymphomatosis. Because lymphomatosis is a condition peculiar to the avian family, these findings and conclusions have no apparent direct application to the etiology of human arteriosclerosis.

Mr. Berley Winton, director of the United States Regional Poultry Research Laboratory, cooperated in fostering this international research program. Mr. Charles Jarvis made the photomicrographs. Mrs. G. Strickland, Miss M. Long and Miss M. Littlefield gave technical assistance.

SCORBUTIC ARTHROPATHY IN THE GUINEA PIG

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THE PATHOLOGIC defects which develop in the bones in acute and chronic scurvy have been the subject of numerous studies, based on both human and experimental material.[1] It was first suggested [2] and then proved experimentally [3] that a deficiency of ascorbic acid (vitamin C) induces pathologic changes not only in the osseous structures but also in all the tissues of mesenchymal origin throughout the body. Visceral lesions of scorbutic animals have also been described by numerous investigators. It seems probable, however, that many if not most of the visceral changes are due to the inanition and dehydration which always accompany the advanced stages of the scorbutic state rather than to ascorbic acid deficiency per se.

Despite the fact that from the time of the first descriptions of this disease the joints have been known to be involved in scurvy, relatively few well documented anatomic studies of the changes taking place in the articulations are available. Rinehart and co-workers [4] described the

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The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

From the Army Medical Nutrition Laboratory, Chicago (Dr. Pirani, Captain Bly and Mr. Sutherland), and the Department of Pathology, University of Illinois College of Medicine (Dr. Pirani).

1. (*a*) Aschoff, L., and Koch, W.: Skorbut: Eine patologischanatomische Studie, Jena, Gustav Fischer, 1919. (*b*) Hess, A. F.: Scurvy, Past and Present, Philadelphia, J. B. Lippincott Company, 1920. (*c*) Höjer, J. A.: Acta pædiat. (supp.) **3**:8-278, 1924. (*d*) Wolbach, S. B., and Howe, P. R.: Arch. Path. **1**:1, 1926. (*e*) Dalldorf, G.: J.A.M.A. **111**:1376, 1938. (*f*) Meyer, A. N., and McCormick, L. M.: Studies on Scurvy, Stanford University, Calif., Stanford University Press, 1928. (*g*) Banks, S. W.: J. Bone & Joint Surg. **25**:553, 1943.

2. Aschoff and Koch.[1a] Höjer.[1c]

3. Wolbach, S. B.: Am. J. Path. (supp.) **9**:689, 1933.

4. (*a*) Rinehart, J. F.: Ann. Int. Med. **9**:671, 1935. (*b*) Rinehart, J. F.; Connor, C. L., and Mettier, S. R.: J. Exper. Med. **59**:97, 1934.

articular lesions of scorbutic guinea pigs, emphasizing that the lesions were similar to those usually found in human material in cases of rheumatoid arthritis. In the opinion of these investigators, the similarity was more striking in those animals in which infection was artificially imposed on a state of partial or chronic scurvy. Successive studies have stressed particular aspects of the changes taking place in and about the joints of guinea pigs in acute and chronic scurvy.[5]

The present investigation was undertaken with the purpose of studying further the nature of scorbutic arthropathy in the guinea pig, emphasis being placed on the course of this condition as shown by the histologic pictures of acute and particularly of chronic lesions, on the role of hemorrhage and of mechanical stress, and finally on the effect of ascorbic acid administered to animals with well advanced lesions. Another objective of this study was to evaluate the importance of ascorbic acid deficiency as a possible factor in the genesis of arthritis of the rheumatoid type. In addition, a more complete working knowledge of the changes of scorbutic arthropathy was a necessary preliminary step toward future studies in which other stresses would be imposed on conditions of ascorbic acid deficiency.

METHODS

Three groups of young guinea pigs, totaling 64, were used for the first part of this study. Of these, 54 weighed between 175 and 250 Gm. and 10 between 450 and 550 Gm. at the start of the experiment. They were offered three different scorbutogenic diets according to the formulas suggested by Rinehart, Connor and Mettier[4b] and by Crampton[6] with minor modifications, and a commercial chow,[7a] to which 1.5 per cent yeast was added.[7b] The amounts of ascorbic acid present in the three diets as determined by chemical analysis[8] were minimal, corresponding to daily intakes of 0.05 to 0.08 mg., compared with the normal requirement for young growing guinea pigs of approximately 2.0 mg. per day The results obtained with

5. (*a*) Schultz, M. P.: Arch. Path. **21**:472, 1936. (*b*) Ham, A. W., and Elliott, H. C.: Am. J. Path. **14**:323, 1938. (*c*) Mouriquand, G., and Dauvergne, M.: Presse méd. **46**:1081, 1938; (*d*) J. méd. franç. **27**:411, 1938. (*e*) Mouriquand, G.; Dauvergne, M.; Tête, H., and Edel, V.: Compt. rend. Acad. d. sc. **210**:515, 1940. (*f*) Mouriquand, G.: Vitamines et carences alimentaires, Paris, Albin Michel, éditeur, 1942.

6. (*a*) Crampton, E. W.; Collier, B. C.; Woolsey, L. D., and Farmer, F. A.: Science **100**:599, 1944. (*b*) Crampton, E. W.: J. Nutrition **33**:491, 1947.

7. (*a*) Purina® rabbit chow supplement, supplied by the Ralston Purina Company, St. Louis, Mo. Different batches of purina® rabbit chow used in other experiments were found to contain amounts of ascorbic acid as high as 10 to 15 mg. per hundred grams. This diet as such, therefore, should be used for experimental study of ascorbic acid deficiency only after analysis. The chow used in this experiment contained only 0.3 to 0.4 mg. per hundred grams. (*b*) Reid, M. E.: Biol. Symposia **12**:373, 1947.

8. Roe, J. H., and Kuether, C. A.: J. Biol. Chem. **147**:399, 1943.

the three diets used were essentially alike in regard to the development of scurvy and the pathologic changes induced. For the purposes of this study, therefore, no subdivision of the animals in groups according to diet was considered necessary. The animals were kept in single cages, with food and water fed ad libitum. In addition to the diet, all the animals received dry hay (swamp grass) for roughage, and vitamins A, D and E (tocopherol) in adequate amounts once weekly.[4b] Twenty-six guinea pigs (acute or total scurvy) received no supplement of ascorbic acid, 20 (chronic, low grade scurvy) 0.2 mg. per day and 18 (controls) 2 to 4 mg. per day of ascorbic acid or fresh lettuce. The ascorbic acid was administered orally every other day. In the control group no significant variations were noted either in the growth curves or in the clinical behavior of the animals fed the different amounts of ascorbic acid or excess lettuce. Weights were recorded twice weekly routinely but daily in the critical phase of the disease. Observations were made on the condition of the animals and particularly on the articulations at least twice weekly. To study the progress of the lesions, the animals were killed at different intervals of time ranging from one to nineteen weeks (chronic scurvy) from the beginning of the experiment. A complete autopsy was performed on every animal. Tissues were fixed in Zenker's solution with acetic acid replaced by solution of formaldehyde U. S. P. Bones were decalcified by the formic acid–citrate method, embedded in celloidin (concentrated pyroxylin) and cut at 10 microns. Multiple sections of the knee joints and one section of a few ribs were examined in the case of each animal. In addition, wrist, elbow, shoulder, ankle, hip and vertebral joints were examined in animals selected from each group. Sections were routinely stained with hematoxylin (Ehrlich's)-eosin. In selected cases, Mallory's stain for connective tissue (Heidenhain's azocarmine modification), Van Gieson's stain, Foot's reticulum stain, Hotchkiss' periodic acid routine and Mallory's phosphotungstic acid-hematoxylin stain were used. The degrees of severity of the lesions observed in and around the knee joint and considered characteristic of the scorbutic process were subjectively graded as slight, moderate, moderately severe and severe (from 1 plus to 4 plus) and tabulated in order better to evaluate the evolution of the pathologic changes.

RESULTS

Clinical Observations.—The first evidence of joint involvement became manifest about the end of the second or at the beginning of the third week in the totally scorbutic guinea pigs and one to two weeks later in the partially scorbutic animals. It usually appeared first at the knees and consisted of enlargement of the ends of the bones forming the joint, subcutaneous hemorrhages and progressively increasing tenderness in the same areas. The involved joints became swollen, and the animals had a progressively more difficult and painful gait. Healthy guinea pigs usually maintain a position of semiflexion of the knees (in angles of approximately 90 degrees) and slightly less flexion of the elbows (100 to 110 degrees). In the scorbutic animals the flexion, especially of the knees, became more acute (to 60 to 45 degrees) with progress and chronicity of the disease. Passive extension became progressively more difficult until there was complete immobilization of the joint in a position of sharp flexion. Similar changes took place in wrists and ankles, accompanied by a varying degree of fixation or ankylosis. The involvement of

hip and shoulder joints appeared to be considerably less severe, while that of the small carpal and tarsal joints did not lend itself to an accurate clinical evaluation. The vertebral column became gradually more rigid with either disappearance or exaggeration (accentuated kyphosis or hunching) of the normal curvatures. As a result, the scorbutic animal assumed a hyperflexed, hunched, rigid appearance, with voluntary motion reduced to an absolute minimum. There was evidence in some animals at least that the mandibular-zygomatic joints were also involved by the scorbutic process. This involvement probably was responsible, together with the loosened and apparently painful condition of the teeth, for the increasing difficulty in mastication.

In addition to the delayed appearance of pathologic changes and the prolonged course of the disease, the clinical picture of the chronically scorbutic guinea pigs presented some other features worth mentioning. The degree of ankylosis in general eventually became more severe than that noted in terminal acute scurvy (three to four weeks), but after having reached a maximum at about the twelfth week it remained stationary. In a few animals a mild "paradoxical" improvement was noted in the condition of the joints after the thirteenth and fourteenth week. Hemorrhagic manifestations about the joints, which reached a maximum between the sixth and the ninth week, diminished in intensity, as a rule, and in many cases ceased almost entirely after the twelfth week. Subcutaneous nodules measuring up to 2 to 4 mm. in diameter, at first semifirm in consistency and markedly hyperemic, later firmer and less congested, were observed in several animals. These nodules were located on the ulnar side of the wrist and near the small carpal and tarsal joints, particularly where enlarged bone structures and the skin were in close apposition. In some of the animals with advanced chronic scurvy, bony or cartilaginous spurs projecting under the skin could be detected, especially lateral to the knee joint.

Pathologic Changes in and About the Knee Joint.—In a normal guinea pig microscopic examination of a longitudinal section through the middle of a knee joint discloses a moderately cellular, smooth articular cartilage and the presence of cruciate ligaments. At the site of insertion of the cruciate ligaments the articular cartilage either is absent or presents transitional features leading to dense fibrous connective tissue. Cuboidal synovial lining is limited to the lateral recesses of the joint, while the more centrally located subsynovial fat pads are lined by flattened cells. Occasionally synovial villi are present in the lateral recesses and "piling up" of synovial lining cells is also seen.

The different pathologic changes which occur in and about the joints during the course of the disease will be considered separately.

Intra-articular hemorrhages were almost always seen in sections taken from animals that died or were killed during the hemorrhagic

phase of the disease, i. e., in the fourth week in acute scurvy and between the sixth and the ninth week in chronic scurvy. The intensity of the hemorrhages, however, varied considerably in different animals killed at the same time, and also appeared to be more severe in the totally than in the partially scorbutic animals (fig. 1). Part of the hemorrhage was quickly reabsorbed, and the corpuscular elements were rapidly destroyed. Fibrin was precipitated in the joint space, where at times it persisted for

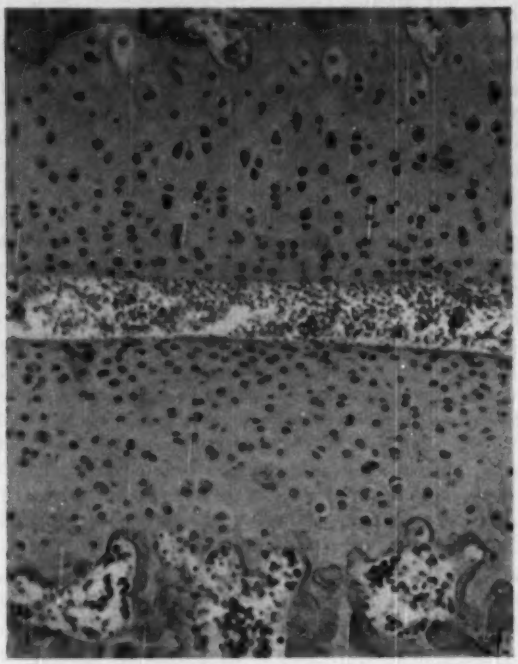

Fig. 1.—Recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid; × 150 (approx.). (Armed Forces Institute of Pathology negative 218409-17.)

several weeks, slowly undergoing organization (figs. 2 and 3). The fibrin present was often abundant, forming dense brilliant eosinophilic masses in the lateral recesses or a thin layer covering the articular surfaces. Its morphologic appearance and the results obtained with special stains (such as phosphotungstic acid–hematoxylin) made identification of this material as fibrin reasonably certain. Organization of the hemorrhage was already under way in the acutely scorbutic animals at the time

of death, and was completed about the eighth or the ninth week in the guinea pigs with chronic scurvy. The fibroblasts which were responsible for this process originated either from the subsynovial connective tissue or, more probably, from modified synovial lining cells. Evidence in favor of the latter interpretation was the fact that organization of the fibrin masses often assumed a pattern which simulated synovial proliferation with formation of atypical villi. Typical or true synovial hyperplasia with formation of villi was observed in a relatively small number of cases in a more advanced stage of the disease (fig. 4). This process

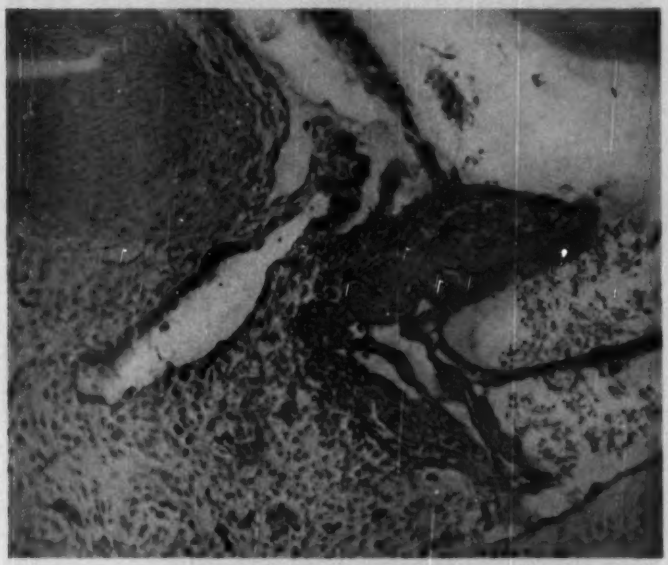

Fig. 2.—Organization of a recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid. Note the degenerating blood corpuscular elements, the masses and strands of fibrin and the proliferation of fibroblasts; × 120 (approx.). (Armed Forces Institute of Pathology negative 218409-13.)

appeared to represent a reaction of the synovial cells to hemorrhagic material acting as a foreign body in the joint space. Even in the more chronic cases the connective tissue resulting from organization of the hemorrhage did not appear to be mature. Fibroblasts often maintained an immature appearance, failing to evolve to fibrocytes, and the intercellular substance could be shown to contain fairly abundant argentaffin fibrils (reticulum) but little or no mature collagen as revealed by Van Gieson's stain.

Simultaneously with the occurrence and organization of intra-articular hemorrhages, considerable fibroblastic proliferation took place in the subsynovial tissue, especially in the fat pads and the periarticular tissue. Although fairly extensive hemorrhages were often noted in these areas, it was difficult to evaluate their role in determining the final pathologic picture. Accumulations of fibrin were never observed in these regions, and also striking was the almost total absence of blood pigment–laden macrophages in all stages of the disease. In the most severe cases,

Fig. 3.—Knee joint showing scorbutic arthropathy in the acute stage; ×27. The anterior ("extensor") synovial pad (at left) shows considerably less change than the posterior ("flexor") one (at right). Note the abundant hemorrhagic, serofibrinous exudate in the joint space and the typical scorbutic changes in the bones. (Armed Forces Institute of Pathology negative 218407-17.)

fibroblasts entirely replaced the adipose tissue of the synovial pads which, as a result, became considerably larger and often entirely filled the recesses of the joint space. Fibroblastic proliferation, as a rule, was considerably more marked in the anterior than in the posterior synovial pad (fig. 3). This difference may have been caused by different degrees of hemorrhage per se in the two pads but more likely was the result of

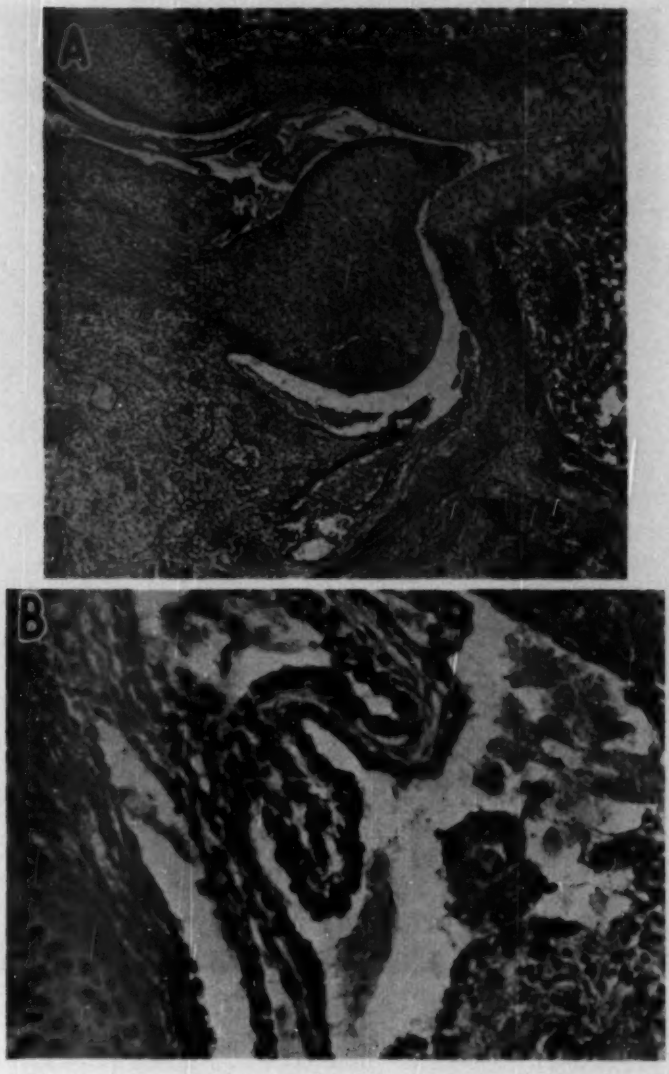

Fig. 4.—*A*, flexor recess of a knee joint in a subacute stage of scurvy, showing true synovial proliferation and large vascular spaces in the young fibrous connective tissue underneath the synovia; ×58. *B*, detail of *A* illustrating "true" synovial proliferation; ×250. Note the regular lining of synovial cells on newly formed villi.

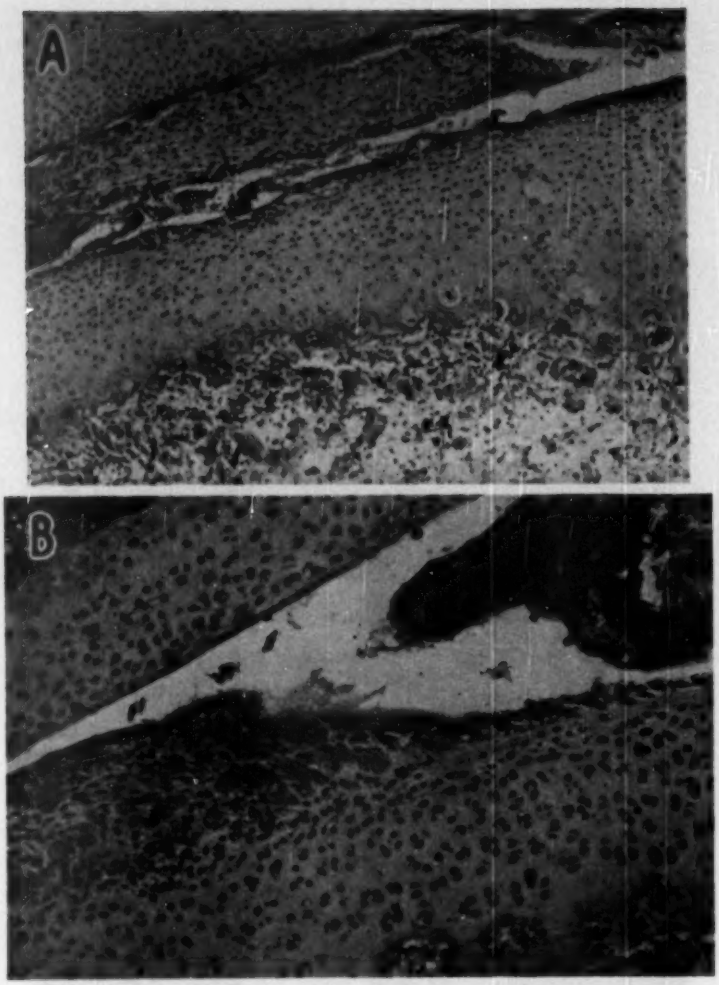

Fig. 5.—*A*, patellar-femoral space in late acute scurvy; ×91. Note the presence of fibrin and erythrocytes together with extensive pannus formation and slight regressive changes of the articular cartilage. (Armed Forces Institute of Pathology negative 218407-2.) *B*, patellar-femoral space in chronic scurvy; × 162. A large mass of fibrin is undergoing organization. The articular cartilage presents superficial vascularization, while the cartilage cells assume the appearance of fibroblasts. (Armed Forces Institute of Pathology negative 218407-5.)

different degrees of mechanical stress (compression) either alone or combined with increased hemorrhage. Because of the normally flexed position of the knee joint in the guinea pig, it seems probable that much of the body weight rests on the posterior (flexor) rather than on the anterior (extensor) aspect of this articulation.

In some animals the proliferating connective tissue (pannus) covered the articular cartilage surfaces. Often, however, in spite of the close apposition of intra-articular connective tissue and articular cartilage, a

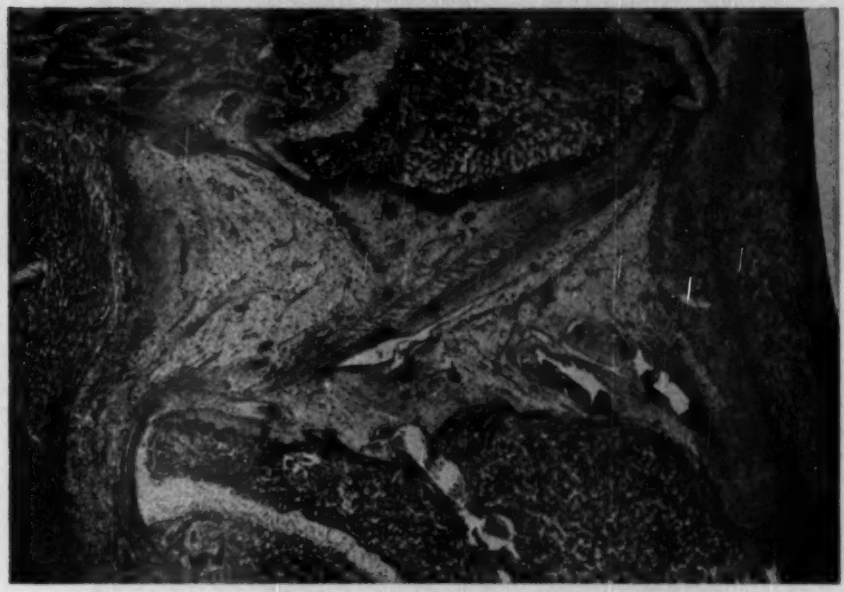

Fig. 6.—Knee joint in chronic scurvy; ×20. There is fibrous ankylosis with partial destruction of the articular cartilage. The entire joint cavity is filled by young proliferating connective tissue. Fibrosis of the periarticular tissue is severe on both the anterior and the posterior aspect of the knee and is continuous with the thick subperiosteal layer of fibrous tissue.

distinct demarcating line between the two structures could still be made out (fig. 5 *A*).

The articular cartilage was studied with particular attention in an attempt to detect changes either in the matrix or in the cells. In the areas where newly formed pannus was in close apposition, the cartilage cells occasionally showed retrogressive changes, assuming the appearance of fibroblasts. In some specimens, newly formed capillaries appeared in the

superficial layers of the cartilage (fig. 5*B*). As a rule, the matrix of articular cartilage was more abundant and stained more darkly than did the matrix of epiphysial cartilage. This staining difference was observed both in the control and in the scorbutic animals but often was more obvious in the latter, indicating a different chemical or physical state of the matrix in the two types of cartilage, which was accentuated by a condition of ascorbic acid deficiency. However, no definite evidence of immaturity was observed by the use of either routine or special stains (Hotchkiss, Mallory's or Van Gieson's). In addition, the matrix of the articular cartilage, both in the control and in the scorbutic animals but especially in the latter, was stained more intensely in the deeper than in the superficial layers, irrespective of the stain used. This finding is difficult to interpret and may indicate either aging or abnormality of the deeper layers of cartilage. No typical fractures were observed in the articular cartilage, but occasionally this structure showed depressed areas, especially in the animals with chronic scurvy, in which the epiphysial bone contained few or no trabeculae. Evidence of excessive and irregular cartilage formation was found not uncommonly at each end of the epiphysial line, but it was rare and less obvious in the articular cartilage. In most instances the articular cartilage showed only minor changes in spite of the considerable amount of fibrous tissue within the knee joint. In 1 animal complete fibrous ankylosis was associated with partial destruction of the articular cartilage (fig. 6).

Considerable fibrosis was observed in the periarticular areas, especially in chronic scurvy. Here again the fibrous tissues appeared cellular and immature, with a limited amount of collagen but with numerous, often distended, thin-walled vascular spaces. Fibrosis was particularly prominent in the flexor aspect of the leg in the popliteal region and adjacent tissues. Where seen, it was clearly distributed according to lines of greater mechanical stress—for instance, along tendons and at muscle insertions. In the advanced lesions it was continuous with the proliferating connective tissue of the synovial pads and the joint cavity and especially with the actively proliferating subperiosteal layer of connective tissue along the diaphysis. This frankly thickened periosteum was the result, first, of hemorrhages which had undergone organization and, second, of a considerable proliferation of osteoblasts from the adjacent bone (fig. 7). These osteoblasts appeared immature, had lost their property of producing bone and gradually had fused with the surrounding mass of fibroblasts. This process could also be observed around the cortex of the epiphyses and occasionally was of considerable magnitude (fig. 8*A*). Therefore it appeared that some of the "fibroblastic" proliferation around the joint was actually due to multiplication of osteoblasts originating from the epiphysial bone. In a few specimens,

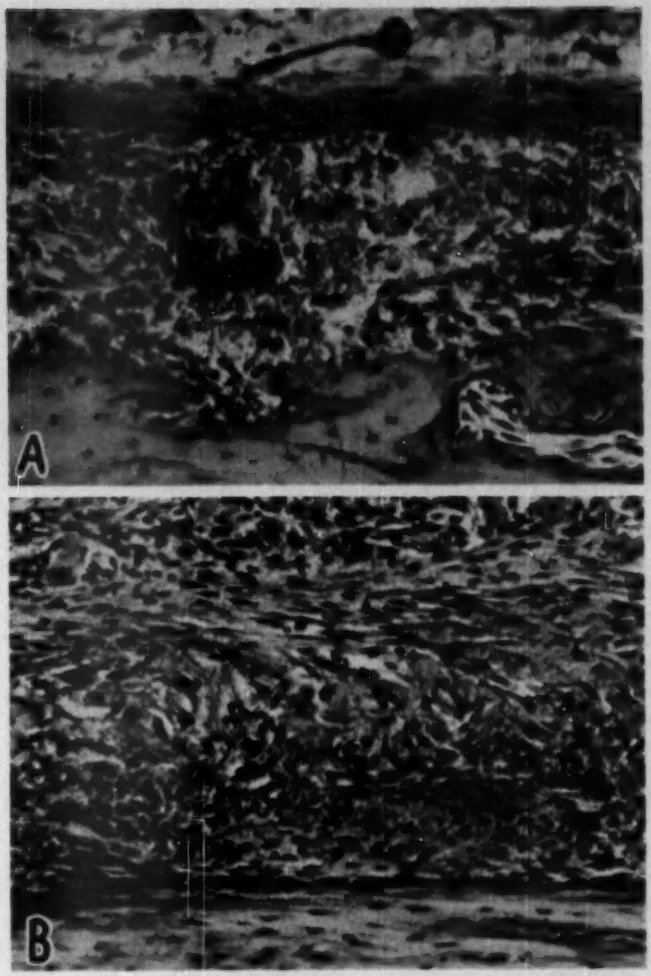

Fig. 7.—*A*, subperiosteal hemorrhages with separation of periosteum (above) and osteoblastic and fibroblastic cellular proliferation in acute scurvy; ×250. *B*, subperiosteal fibrosis with active fibroblastic proliferation; ×250. The periosteum is still recognizable above.

plaques of cartilage cells were also noted at a considerable distance from the joint space and even outside the joint capsule in the popliteal space (fig. 8*B*). It was impossible to say whether they represented a true heterotopic formation of cartilage or were cartilage cells displaced either from the articular surface or from the semilunar cartilages.

Throughout this impressive fibroblastic proliferation that took place both within and around the joint space, inflammatory cells were strikingly absent except for occasional lymphocytes and macrophages in the perivascular spaces.

Histologically, the subcutaneous nodules mentioned previously consisted of fibroblasts either irregularly distributed or arranged in whorls (fig. 9). Although there was a little immature collagen between the cells, there were no areas of necrosis. Fibrinoid degeneration was not observed. Nodules, however, were not examined microscopically in the early stages of development, when grossly they appeared markedly hyperemic and soft in consistency. Their typical position was outside a joint where the enlarged ends of the bones were closely apposed to the overlying skin. Hemorrhages, frequently seen in these regions, and the movement of the bone against the subcutaneous tissue were apparently the factors responsible for the formation of these structures.

Pathologic Changes in Joints Other Than the Knee.—The lesions observed in other joints were essentially similar to those described for the knees. The process was severe at wrists, ankles and elbows but considerably less, as a rule, at hips and shoulders. In the spine the costovertebral articulations were also involved, often severely, while the intervertebral articulations showed only slight changes in the few specimens that were studied. In addition, active fibrocellular proliferation was noted about the spinous processes and the longitudinal ligament, explaining the rigidity of the vertebral column observed clinically in many of these animals. The zygomatic-mandibular joint was examined in 2 guinea pigs with chronic scurvy. Typical scorbutic changes were observed both in the mandible and in the zygomatic bones. In addition a moderate degree of periarticular fibrosis with occasional hemorrhages was noted. The joint proper did not appear to be involved. These findings were believed to be important in accounting for the difficult and painful mastication.

In spite of the considerable differences in the severity of the scorbutic changes in different joints and also between two symmetric joints, it is clear that this process affects practically every articulation in the body. The difference in severity of the lesions appeared to depend mainly on the degree of mechanical stress and hemorrhage in different areas. At the knee the extent of the articular and periarticular involvement was somewhat proportional to the osseous changes in the adjacent bones.

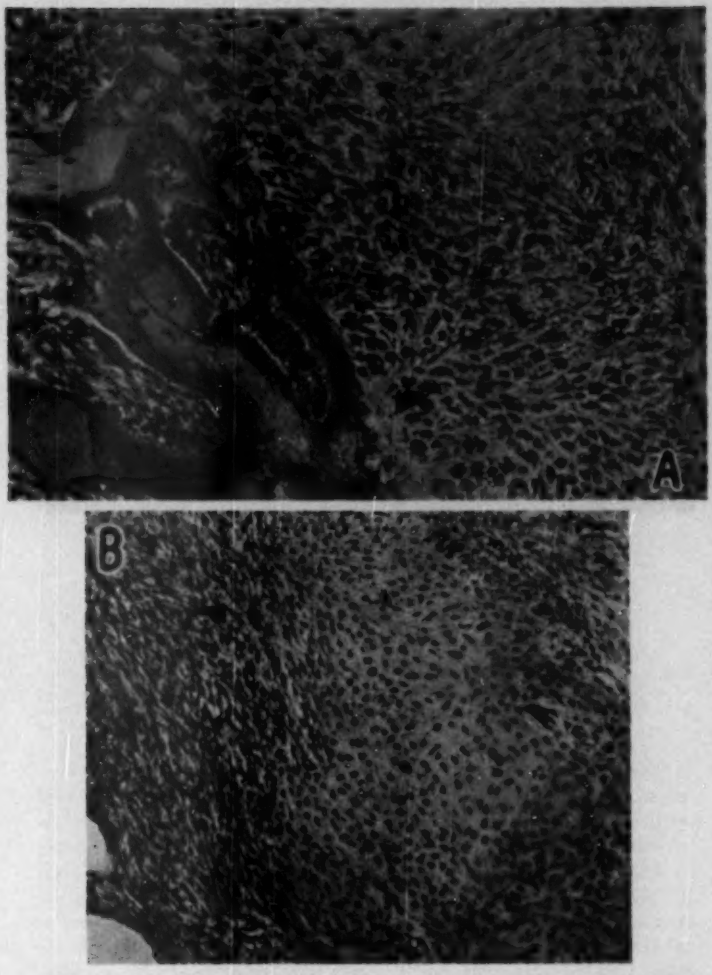

Fig. 8.—*A*, chronic scurvy; × 350 (approx.). Osteoblasts are proliferating from the epiphysis of the femur into the periarticular fibrous connective tissue. (Armed Forces Institute of Pathology negative 218409-7.) *B*, chronic scurvy; × 200 (approx.). There is heterotopic formation of cartilage in periarticular fibrous connective tissue. (Armed Forces Institute of Pathology negative 218409-11.)

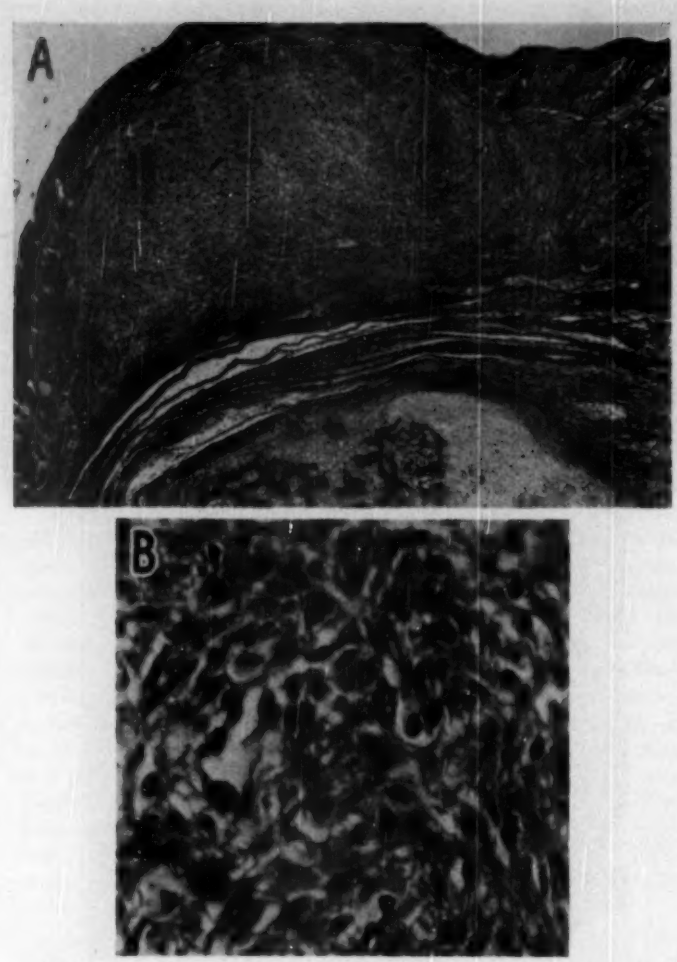

Fig. 9.—*A*, chronic scurvy; ×27. The subcutaneous fibroblastic nodule at the radial aspect of the wrist is in close apposition with the bone. *B*, detail of *A* demonstrating the arrangement of the fibroblasts; ×500.

However, this was not generally true for other joints. For example, extensive changes may be found in the small carpal or tarsal joints without lesions of a corresponding severity being present in the small bones. It appeared, therefore, that in these regions the articular lesions were largely independent of the bony alterations. However, since the severity of the osseous changes of scurvy is known to be dependent in part on the activity of the epiphysial plates [9] and, as a rule, a more severe arthropathy is found in those joints which are formed by the more actively growing bones (i. e., the knee), the concept that there is a partial parallelism between articular and osseous lesions must be accepted.

Course of Scorbutic Arthropathy.—The evolution of some of the more important pathologic changes occurring in the joints over the experimental period considered is summarized in figure 10.

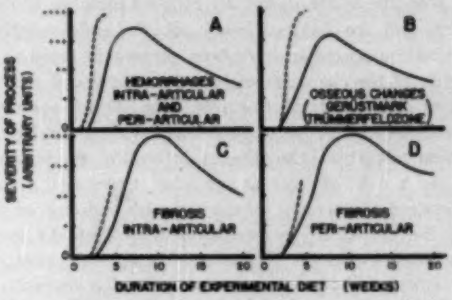

Fig. 10.—A schematic presentation of the changes occurring in the joints of scorbutic guinea pigs. Acute scurvy is represented by a broken line. The guinea pigs showing acute scurvy had been restricted to a diet practically free of ascorbic acid. Chronic scurvy, represented by an unbroken line, was produced by restricting the animals to an intake of ascorbic acid of 0.2 mg. per day.

Intra-articular and periarticular hemorrhages, which are considered together (fig. 10 *A*), developed earlier and were more severe in the acutely scorbutic than in the chronically scorbutic animals. In the latter group these hemorrhagic manifestations reached a maximum between the sixth and the ninth week to decline progressively to only slight or moderate intensity between the fourteenth and the nineteenth week. It is interesting to note that most of the guinea pigs of the group with chronic involvement either died or appeared critically ill between the eighth and the tenth to eleventh week. Those which survived this period appeared to adapt themselves to some extent to the experimental conditions and showed slight gain in appetite and weight, improved appearance and activity, and possibly slightly increased mobility of joints,

9. Glasunow, M.: Virchows Arch. f. path. Anat. **290**:120, 1937.

although this may have been due merely to a disappearance of pain and tenderness. The reason for this decline in intensity of the hemorrhagic manifestations was not clear. However, the number and the size of the capillaries and vascular spaces in the areas of fibroblastic proliferation were considerably reduced after the eleventh week. In addition, with the progress of the disease, the areas of fibrocellular proliferation appeared more dense and, although only small amounts of collagen were formed, the walls of the capillaries were probably better supported by the adjacent tissue. Changes, such as fibrosis and at times hyalinization of the smaller arteries, or endothelial hyperplasia and thickening of the capillary wall, were observed in many cases but apparently were too irregular and inconsistent to have played an important role in diminishing the intensity of the hemorrhagic manifestations. Other factors which may have been of importance in this phenomenon were the reduction of mechanical stress on the joint area due to the extremely reduced mobility of the limbs in chronic scurvy and the reduced blood volume, and probably sluggish circulation, which appeared to have been present in these animals.

The severity of the characteristic osseous changes of scurvy such as the *Gerüstmark* (rarefied trabeculae and loose fibrous marrow) and the *Trümmerfeldzone* (zone of dense abnormal calcified cartilage) followed a pattern similar to that of hemorrhages during the course of the disease (fig. 1 *B*). As a rule, at least at the knee, there was a considerable parallelism between the severity of the articular and that of the osseous changes. In the chronically scorbutic animals after the twelfth week both the *Gerüstmark* and the *Trümmerfeldzone* underwent resorption, to disappear almost entirely in many cases after the sixteenth week. It was interesting to note that resorption of the abnormal trabeculae (*Trümmerfeldzone*) and of the fibrous tissue (*Gerüstmark*), together with the fibrin and amorphous bone matrix present in this region, had taken place in the absence of any detectable activity either of osteoclasts or of macrophages. In this respect the resorptive process was similar to, although more complete than, that of the intra-articular fibrosis present in the same animals. While forming of new bone at the epiphysial plate had ceased almost entirely, new, apparently defective bone was formed in the cortex of the diaphysis, which appeared then considerably thickened. Control guinea pigs of the same age group, which had been under experimental observation for corresponding periods of time, showed persistent bone formation at the epiphysial plate and a thinner cortex. It appeared, therefore, that chronic severe ascorbic acid deficiency had induced an accelerated aging in the process of bone formation as evidenced by the fact that in the guinea pig epiphysial bone formation ceases and periosteal bone formation may continue, even in excess of normal, when the ascorbic acid intake is approximately one tenth of what is considered the normal requirement. These findings can be

explained in part by the fact that the untoward effects of ascorbic acid deficiency become less apparent as the growth rate declines, or are retarded as a result of other physiologic inadequacies resulting from the deficiency.

Intra-articular and periarticular fibrosis (fig. 10 *C* and *D*) was also considerably more severe in the chronically scorbutic than in the acutely scorbutic animals. It was first noticed about a week after the onset of the hemorrhagic manifestations and reached its maximum between the eighth and the twelfth week. It then declined more in the intra-articular than in the periarticular region. Within the joint space the fibrin was reabsorbed. Synovial proliferation was progressively less notable, and there was reappearance of adipose tissue in the synovial pads. In the periarticular region, however, during this period the fibrosis was reduced only slightly, so that functional improvement, as a rule, was limited and the fixed position of the knee joint was not appreciably changed. This condition can be called pseudoankylosis in contrast with true fibrous ankylosis, which occurs at the height of the disease.

Role of Mechanical Stress.—The importance of mechanical force in determining and aggravating the scorbutic lesions of the bones and adjacent tissues is well known. To investigate this point further, with special regard to the joint changes, the left leg of each of 10 young adult guinea pigs weighing between 450 and 550 Gm. was immobilized in an extended position in a plaster of paris cast with a technic similar to that used by Follis.[10] With this method the effect of weight and motion on the knee joint was removed. The animals were restricted to a scorbutic regimen for thirty days. Seven received no supplement, and 3 were controls. The diet used for this as well as for the succeeding group of guinea pigs was Rockland® rat pellets, supplemented by dry hay, yeast, rutin and fat-soluble vitamins. This diet was found later to contain ascorbic acid in amounts corresponding to an intake of approximately 0.2 to 0.4 mg. per day per animal. As a result the gross and the microscopic changes observed in this group of animals were those of "chronic" rather than of "acute" scurvy.

The extremity to which a cast had been applied showed at autopsy a varying but often considerable degree of muscular atrophy and in many cases slight to moderate subcutaneous edema, indicating a corresponding degree of circulatory impairment. Knee joints only were examined. Histologically, the results of Follis (i. e., considerably less severe or no scorbutic lesions in the bones of the immobilized leg) were fully confirmed in only 1 of 7 scorbutic animals studied. In a few animals the immobilized knee had less severe changes in the bones than the opposite, free extremity. In other cases, however, scorbutic changes

10. Follis, R. H.: Arch. Path. **35**:579, 1943.

were at least as severe in the immobilized as in the free extremity. This discrepancy of results can be explained in part by the fact that to 3 of the animals casts were applied ten days after the beginning of the scorbutic regimen. In all scorbutic animals, including those in which only minimal scorbutic changes were seen in the cast-encased legs, the incidence and the degree of the hemorrhagic manifestations were not modified. Hemorrhages, in fact, appeared to be more severe in the immobilized than in the free joint and to involve equally the flexor and the extensor aspect of the articulation. As a result, considerable fibroblastic proliferation had taken place in these joints. In most of the immobilized joints the intra-articular changes were at least as severe as, and did not differ considerably from, those found in the "free" joints of scorbutic animals. The periarticular fibrosis, however, was considerably less severe and more evenly distributed between the extensor and the flexor aspect of the cast-encased knee. In the control animals, hemorrhages were minimal or absent in both legs.

These results seem to indicate that hemorrhage plays a dominant role as far as the intra-articular lesions of scorbutic arthropathy are concerned. Mechanical stress, on the other hand, appears to be a determining factor in the considerably more abundant periarticular fibrosis, which is usually observed on the flexor aspect of the nonimmobilized joint. When the normal position of flexion is changed to one of forced extension and mechanical stress is removed, the periarticular fibrosis is distinctly reduced on the flexor aspect and slightly increased on the extensor aspect of the knee joint.

Effect of Ascorbic Acid Administered for Scorbutic Arthropathy.—A preliminary study to investigate the effect of administered ascorbic acid on preexistent articular lesions of scurvy was made on a group of 8 young adult guinea pigs. These animals were restricted to a scorbutogenic diet, including Rockland® rat pellets, for thirty days. At the end of this period 4 animals were given 0.4 mg. of ascorbic acid per day, and 4, 2 mg. per day for periods increasing from one to twenty-five days. Knees and ribs were examined histologically. The clinical and pathologic observations on this small group of animals indicated that much of the intra-articular and some of the periarticular fibrosis disappears after treatment. In the joint space, however, a small amount of fibrin may persist even after twenty-five days of rehabilitation. In addition, some of the periarticular fibrotic changes persisted throughout the experimental period, especially on the flexor aspect of the joint and in the adjacent muscles. This fibrous connective tissue became more mature and fairly abundant collagen was formed. The *Trümmerfeldzone* and the *Gerüstmark* were slowly reabsorbed and a limited number of new bone trabeculae were formed at the epiphysial line. Osteoblastic

activity was much greater in the subperiosteal space. In this area the osteoblasts which appeared as fibroblasts or immature mesenchymal cells in the scorbutic state reacquired their typical appearance and produced new bone at a remarkably fast rate. In this respect no difference was noted in the animals receiving either 0.4 or 2.0 mg. of ascorbic acid per day. No bone formation was observed in the masses of immature mesenchymal cells of the periarticular tissue. Osteoblastic activity and new bone formation were also limited in amount within the epiphyses.

COMMENT

The results of these experiments clearly indicate that a severe arthropathy develops in guinea pigs subjected to a condition of total or severe partial ascorbic acid deficiency. This disturbance cannot be considered to be a separate entity but is a part of the general manifestations of scurvy. The fundamental features of scurvy, namely, hemorrhages, active proliferation and failure of maturation of mesenchymal cells, and production of scanty ground substance and of immature collagen are all present at one stage or another of scorbutic arthropathy, this disease being obviously dependent on these manifestations.

The observations of other investigators [11] have been in general confirmed and extended in this study. Hemorrhages obviously play a more important role, especially in the earlier stages of the disease, than is admitted by some.[12] It is evident that unless the animals are killed and examined during the hemorrhagic phase of scurvy, the blood extravasated into the joint cavity and in adjacent areas will have been at least partially reabsorbed or organized by actively proliferating mesenchymal cells, and the importance of hemorrhages in initiating and maintaining the arthritic changes may then be underestimated. The importance of this factor is also proved by the fact that the condition of the joints may improve considerably after the hemorrhagic phase of the disease is over. Fibrous ankylosis persists in the chronically scorbutic animals because of the periarticular rather than the intra-articular lesions.

The fact that guinea pigs will adjust to a chronic severe partial ascorbic acid deficiency, probably on the basis of a sharp reduction of metabolism and therefore of ascorbic acid requirements, must be kept in mind in evaluating some of the pathologic changes. This phenomenon had been observed by Mouriquand in "chronic" scorbutic guinea pigs and called *paravitaminose*. According to this investigator, many of the lesions of these animals do not respond to ascorbic acid therapy and can be considered irreversible as measured by clinical observation and roentgenologic studies.

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11. Wolbach and Howe.[1d] Rinehart.[4a] Rinehart and others.[4b] Schultz.[5a] Ham and Elliott.[5b] Mouriquand and Dauvergne.[6c,d] Mouriquand and others.[6e]
12. Meyer and McCormick.[1f] Rinehart.[4a]

The fact that there was no significant inflammatory cell infiltration within and around the joint at all stages of the disease must be stressed. This was also true of 8 guinea pigs in which a severe infectious process, such as bronchopneumonia, was found at autopsy. In these animals, however, the lesions of the joints appeared to be somewhat more severe than in comparable animals at the same stage of scurvy, probably indicating an aggravation of the scorbutic state. It is interesting to note that macrophages were seen only occasionally even around the areas of recent and old hemorrhage. The same applies to osteoclasts in spite of the considerable reabsorption of bone which takes place in chronically scorbutic animals.

Mechanical force (tension and compression) is without doubt important in determining the site and the severity of the articular and periarticular lesions of acute and chronic scurvy. The results obtained in the guinea pigs which had a leg immobilized in a cast are, however, somewhat different and less convincing than those reported by Follis.[10] A difference of experimental technics may have been responsible for this discrepancy. We feel, however, that although mechanical stress is important and is responsible for many of the features of scurvy, its importance should not be emphasized to the exclusion of other factors, particularly hemorrhage.

Some of the features of scorbutic arthropathy bear a resemblance to those of rheumatoid arthritis. We were not impressed, however, by any striking similarity of the two articular disorders when the over-all pathologic picture was considered. Hemorrhagic manifestations are definitely more severe in scurvy, as are the osseous changes. It is true that in occasional animals with chronic scurvy it is difficult to detect changes that are pathognomonic for scurvy. However, other lesions, such as small areas of fibrosis in the marrow and thickening of the periosteal bone, were present in most of these cases. To the best of our knowledge, no such changes have been mentioned as occurring in rheumatoid arthritis in any stage of the disease. Disuse atrophy is generally considered to be the main factor in causing osteoporosis in rheumatoid arthritis and possibly is partially responsible, together with inanition, for the osteoporosis of chronic scurvy. Synovial and subsynovial fibroblastic proliferation with pannus formation is present in both diseases and may lead to fibrous ankylosis. This condition, however, is spontaneously reversible, at least in part, in advanced chronic scurvy and never progresses to bony ankylosis. The fibroblastic proliferation of scurvy, therefore, does not seem to have the destructive properties which, as a rule, are observed in infectious processes and in rheumatoid arthritis. It is probably for this reason that in chronic scurvy with few exceptions the articular cartilage shows mild lesions of a regressive rather than a destructive nature. Although the severity of

scorbutic arthropathy may vary considerably in different joints, the lesions always appear to be in approximately the same stage, contrary to what is generally found in rheumatoid arthritis.

Changes consistent morphologically with fibrinoid degeneration were not uncommonly observed in the areas of mesenchymal cell proliferation especially in acute and chronic scurvy. This lesion was general in distribution and the adjacent cells were never arranged to form granulomatous nodules. The subcutaneous periarticular nodules that are occasionally found in chronic scurvy were also, in our opinion, unlike similarly placed lesions of human patients with either rheumatic fever or rheumatoid arthritis. The similarity of scorbutic arthropathy and rheumatoid arthritis is therefore only superficial. The differences between the two diseases become more apparent when the chronic lesions of scorbutic arthropathy are studied serially and when the entire course of this scorbutic process is reconstructed. Despite these differences, the possibility that partial ascorbic acid deficiency may be a predisposing or a contributing factor in certain forms of human arthritis cannot be ruled out entirely. The recent reports of striking effects of an adrenal cortex steroid (compound E [17-hydroxy-11-dehydrocorticosterone]) on the courses of rheumatoid arthritis and rheumatic fever [13] are of interest in this regard, and since ascorbic acid has been implicated as playing a role in the synthesis of adrenocortical hormones,[14] it would appear possible that ascorbic acid deficiency may play an indirect role in the genesis of these two diseases. Additional work to investigate this possibility is indicated.

SUMMARY

The articular lesions of acute and chronic scurvy were studied in 82 guinea pigs.

Scorbutic arthropathy involves in different degrees all the joints of the body. It is characterized by hemorrhages and fibroblastic proliferation, minor changes of the articular cartilage and striking absence of inflammatory cells. Hemorrhages and mechanical stress are the all-important factors in determining the general picture of scorbutic arthropathy. The incidence and the severity of the hemorrhagic manifestations decline considerably as chronic scurvy advances. Spontaneous improvement of the intra-articular lesions (true ankylosis) also occurs. Periarticular lesions (pseudoankylosis) are less likely to improve spon-

13. Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Proc. Staff Meet., Mayo Clin. **24**:181, 1949. Hench, P. S.; Slocumb, C. H.; Barnes, A. R.; Smith, H. L.; Polley, H. F., and Kendall, E. C.: Ibid. **24**:277, 1949.

14. Giroud, A.; Santa, N.; Martinet, M., and Bellon, M. T.: Compt. rend. Soc. de biol. **134**:100, 1940. Sayers, G.; Sayers, M. A.; Lewis, H. L., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **55**:238, 1944.

taneously or even after therapy. Under the conditions of the experiment the periarticular fibrosis appears to be, for the most part, irreversible and responsible for the persistent functional impairment.

There is only a superficial morphologic similarity between the articular lesions of scurvy and those of either rheumatoid arthritis or rheumatic fever. It is possible, however, that partial ascorbic acid deficiency may be a predisposing or a contributing factor in certain forms of human arthritis.

In this investigation advice and encouragement were given by Dr. R. E. Johnson, technical assistance by Mr. F. Chereso and cooperation in taking many of the pictures by the Armed Forces Institute of Pathology.

MAMMARY GROWTH IN ORCHIDECTOMIZED MICE GRAFTED WITH ANTERIOR LOBES OF HYPOPHYSES AND OVARIES AT VARIOUS AGES

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MICE OF strain A orchidectomized at 3 to 4 weeks of age and receiving ovarian transplants a few days later showed in some instances development of mammary cancers. The tumor incidence was markedly increased if the anterior lobes of the hypophysis were grafted, together with the ovaries.[1] This was presumably due to increased output of estrogen on the part of the ovarian grafts, which were stimulated by the hypophysial transplants. Thus a single transplantation of an endocrine organ may increase the production of hormones to such a degree that neoplastic growth may ensue in certain target organs.[1a, c] This method of producing cancers resembles more closely spontaneous carcinogenesis than does the administration of exogenous extracts or preparations of the glands. If transplantation was performed six months after castration, no mammary cancers occurred in any of the transplant-bearing animals, even if they had received ovaries in combination with anterior lobes of the hypophysis. This observation seemed to indicate that an age factor operates in this type of mammary carcinogenesis as it did in male mice of strain C3H given injections of estrogens at various ages.[2]

The present investigation deals with microscopic observations of the growth response of the noncancerous mammary glands of the transplant-bearing castrates. These observations were thought to give some infor-

From the Snodgras Laboratory, Hospital Division, City of Saint Louis.

The investigation was supported by a research grant from the National Cancer Institute, United States Public Health Service.

1. (*a*) Loeb, L., and Kirtz, M. M.: Am. J. Cancer **36**:56, 1939. (*b*) Loeb, L.; Blumenthal, H. T., and Kirtz, M. M.: Science **99**:230, 1944. (*c*) Loeb, L., in Alexander, J.: Colloid Chemistry, New York, Reinhold Publishing Corporation, 1944, pp. 995-1050. (*d*) Silberberg, R., and Silberberg, M.: Proc. Soc. Exper. Biol. & Med. **70**:510, 1949; **72**:547, 1949; (*e*) Arch. Path. **48**:557, 1949.

2. (*a*) Loeb, L.: Biol. Symposia **11**:197, 1945. (*b*) Silberberg, M., and Silberberg, R.: Arch. Path. **47**:340, 1949; (*c*) Proc. Soc. Exper. Biol. & Med. **69**:438, 1948.

mation concerning the moot question of the mode of action of the hypophysial and ovarian hormones stimulating mammary growth.

MATERIAL AND METHODS

One hundred and sixty-four male mice of the closely inbred strain A, raised in our laboratory and fed a stock diet of commercial chow [3] and water ad libitum, were orchidectomized at the age of 3 to 4 weeks. Eighty-one of these castrates received transplants at the age of 1 month (younger age group), and 83 at the age of 7 months (older age group). The glands transplanted to the individual mice were: (*a*) 4 anterior lobes of hypophyses [3a] (28 mice of each group); (*b*) 2 or 4 ovaries (27 mice of the younger and 26 of the older group); (*c*) 4 anterior lobes of hypophyses together with 2 or 4 ovaries (26 mice of the younger and 29 mice of the older group).

Most of the glands to be grafted were obtained from 2 to 3 month old animals closely related to the recipients, that is, from brothers, sisters or cousins (syngeniotransplantation [4]); 38 mice received glands from less closely related donors (homoiotransplantation). The glands to be grafted were removed from freshly killed animals under sterile precautions and kept in sterile Ringer's solution for a short time, until two subcutaneous pockets had been prepared one on each side of the chest wall of the recipient. The grafts were transferred into these pockets, which were then closed with silk sutures. The animals were allowed to live to the end of their natural life or until mammary cancers or lymphoid tumors developed.

At necropsy the transplants or tissues that appeared to be remnants of transplants were removed and fixed in a 4 per cent solution of formaldehyde together with internal organs, endocrine glands, some bones and mammary glands. Semiserial sections were made and stained with hematoxylin and eosin. Not all the material, however, was used for microscopic examination, since some tissues had undergone autolytic changes. For the present report the mammary glands of 131 animals were studied. Details can be seen in table 1.

MICROSCOPIC CRITERIA OF THE STAGES OF MAMMARY STIMULATION

The stages to be described here were used to characterize the microscopic observations and, in particular, the pattern of growth encountered in the mammary gland. In this type of classification a certain arbitrariness is unavoidable, because there are no sharp lines of demarcation, and gradual transitions occur between the different stages. However, a schematic tabulation may facilitate a comparison of the observations in the various experimental groups.

Resting Condition.—In a lower power field (48 mm. objective, ocular × 10), the number of ducts cut across or lengthwise varies from 2 to 8. These scanty ducts are collapsed, embedded in abundant fat

3. This was purina® laboratory chow B-2362 made by the Ralston Purina Mills, St. Louis.

3a. The hypophysis of the mouse shows two anterior lobes, or segments.

4. Loeb, L.: The Biological Basis of Individuality, Springfield, Ill., Charles C Thomas, Publisher, 1945.

tissue and lined by low cuboidal epithelium. There are no secretory vacuoles in the cells, and the ductal lumens are free of secretions.

Slight Stimulation.—In a low power field as many as 16 sections of elongated or tortuous ducts may be counted. These ducts are accompanied by scanty, loose, vascular connective tissue; they give off smaller branches or show terminal budding. The lining epithelial cells are increased in number and size, both cytoplasm and nuclei undergoing hypertrophy. Here and there a mitotic figure may be seen. There is infolding of the hyperplastic epithelium, with papilliform structures projecting into the lumen. However, alveoli are not present. The ductal

TABLE 1.—*Incidence of Mammary Cancers and of Findings in the Noncancerous Mammary Glands*

Expt. Group	Glands Transplanted to Mice Castrated at 3 Weeks	Mice Receiving Grafts	Mice Subsequently Showing Mammary Cancers			Mice with Nonneoplastic Mammary Glands Which Were Microscopically Examined									
							Mice with Resting Glands			Mice Whose Glands Showed Slight Stimulation			Mice Whose Glands Showed Advanced Stimulation		
			No.	% of Total	Mean Age, Mo.	Total No.	No.	% of Total	Mean Age, Mo.	No.	% of Total	Mean Age, Mo.	No.	% of Total	Mean Age, Mo.
1	Ant. hypophysial lobes at 1 mo..	28	0	0	...	17	10	58.8	17.9 (12-30)*	4	23.5	17.5 (16-19)*	3†	17.7	17.7 (6-19)*
2	Ovaries at 1 mo.....	27	1	4.7	6.5	20	9	34.6	19.0 (11-24)*	7	26.9	16.3 (12-21)*	10‡	38.5	16.7 (7-24)*
3	Ant. hypophysial lobes and ovaries at 1 mo.....	26	6	30.8	12.9 (8-20)*	25	6	32.0	18.5 (15-22)*	4	16.0	20.3 (15-27)*	13§	52.0	15.7 (8-27)*
4	Ant. hypophysial lobes at 7 mo..	29	0	0	...	21	15	71.4	21.2 (15-35)*	4	19.0	16.5 (15-24)*	2	9.5	15.0
5	Ovaries at 7 mo.....	20	0	0	...	22	18	81.8	17.6 (7-26)*	1	4.6	17.0	3‖	13.6	14.0 (12-17)*
6	Ant. hypophysial lobes and ovaries at 7 mo.....	29	0	0	...	20	12	60.0	15.6 (8-24)*	3	15.0	17.2 (14-24)*	5¶	25.0	15.6 (10-22)*

* Age range in months.
† One showed slight alveolar proliferation.
‡ Seven showed advanced alveolar proliferation.
§ Twelve showed advanced alveolar proliferation.
‖ Three showed slight alveolar proliferation.
¶ Two showed moderate alveolar proliferation.

epithelium contains secretory vacuoles, and homogeneous eosinophilic secretions appear in the lumen.

Advanced Stimulation.—In individual mammary glands, as many as 30 and more cross sections of often closely packed, elongated and dilated ducts may be noted. Many new ducts are seen to branch off from the larger ones, and clusters of terminal buds are frequent. The cells of the epithelial lining are hypertrophic and contain secretory vacuoles. Moreover, the epithelium is hyperplastic, and mitotic figures are common. The intraductal papillary projections reach considerable size and may coil. Homogeneous eosinophilic secretions are abundant inside the lumens. In addition, small nodules composed of closely

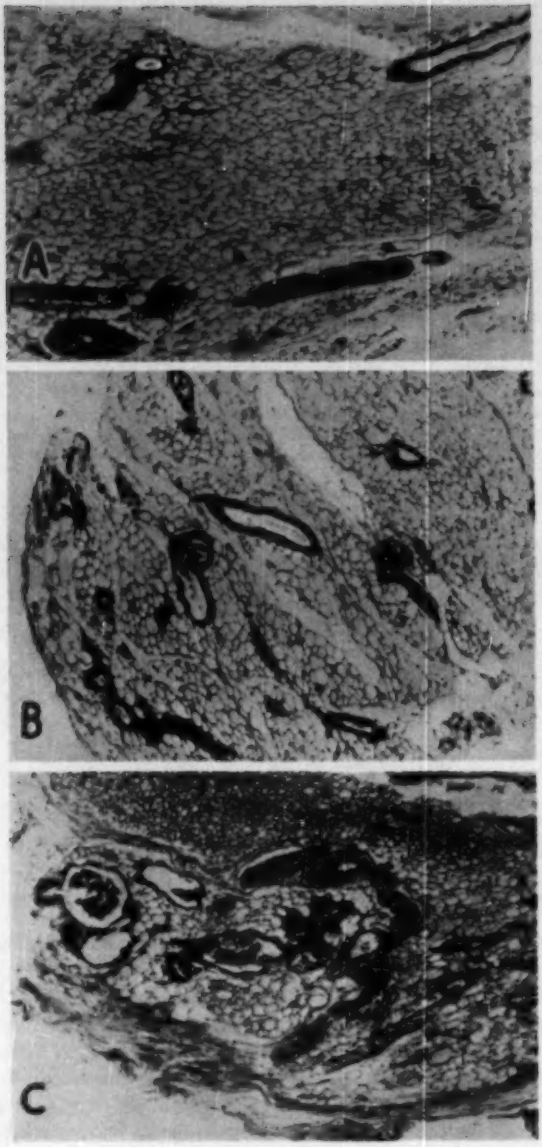

Fig. 1.—The photomicrographs presented in figures 1 to 6, inclusive, show representative sections of the mammary glands of castrate male mice of strain A which had received grafts of endocrine glands at various ages. Magnification, × 65. The sections shown in this figure represent mammary glands of mice each of which received four anterior lobes of hypophyses at the age of 1 month: *A*, mouse 16½ months old; the mammary gland is resting (no effect). *B*, mouse 19 months old; the mammary gland shows ductal growth with secretion (slight effect). *C*, mouse 16 months old; the mammary gland shows ductal growth with secretion and budding and slight alveolar growth (advanced effect).

packed dilated ducts supported by vascular connective tissue and clusters of alveoli grouped around ends of ducts are found. The size of the alveoli and their contents of secretions may vary considerably. In the small ones a lumen may be barely noticeable; others of the newly formed alveoli are distended with secretions.

COMPILATION OF DATA

The microscopic appearances in the mice of the various experimental groups were classified according to the criteria laid down in the preceding section, and are compiled in table 1. The table shows the following data in consecutive vertical main columns: the type of transplant and the age at which the graft was received; the total number of mice in each experimental group; the number of mice which subsequently showed cancer in each group; the number of mice with noncancerous mammary glands which were studied microscopically in each group and the numbers of these animals showing resting, slightly or excessively stimulated mammary glands, respectively. Each main column is divided into subcolumns giving, in addition to the number of mice, the percentage and the mean age at death of all animals in the group. The incidence and the degree of alveolar growth are indicated by symbols pointing to footnotes.

YOUNGER AGE GROUPS

1. *Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Anterior Hypophysial Transplants at the Age of 1 Month (28 Mice).*—No mammary cancer had developed at the time these mice died or were killed and examined. The mammary glands of 17 mice were sectioned (fig. 1). In 10 animals (58.8 per cent) killed or dying at a mean age of 17.2 months, the glands were in a resting state. In 4 animals (23.5 per cent) with a mean age of 17.5 months, there was slight stimulation of the mammary glands, and in 3 animals (17.7 per cent) dying at the mean age of 17.7 months, advanced ductal stimulation and secretion were noted. In 1 of these 3 mice a few alveoli were scattered around in the fat tissue (fig. 1 *C*).

2. *Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Ovarian Transplants at the Age of 1 Month (27 Mice).*—One mouse (4.7 per cent) had a mammary cancer at the age of 6.5 months. The mammary glands of 26 animals were examined microscopically (fig. 2). In 9 of the mice (34.6 per cent), dead at a mean age of 19 months, the mammary glands were resting. Seven animals (26.9 per cent) with a mean age of 16.3 months showed slight mammary growth. Ten other animals (38.5 per cent), dead at a mean age of 16.7 months, disclosed advanced ductal growth with secretion; in 7 of the latter mice, alveolar growth and secretion were observed (fig. 2 *C*).

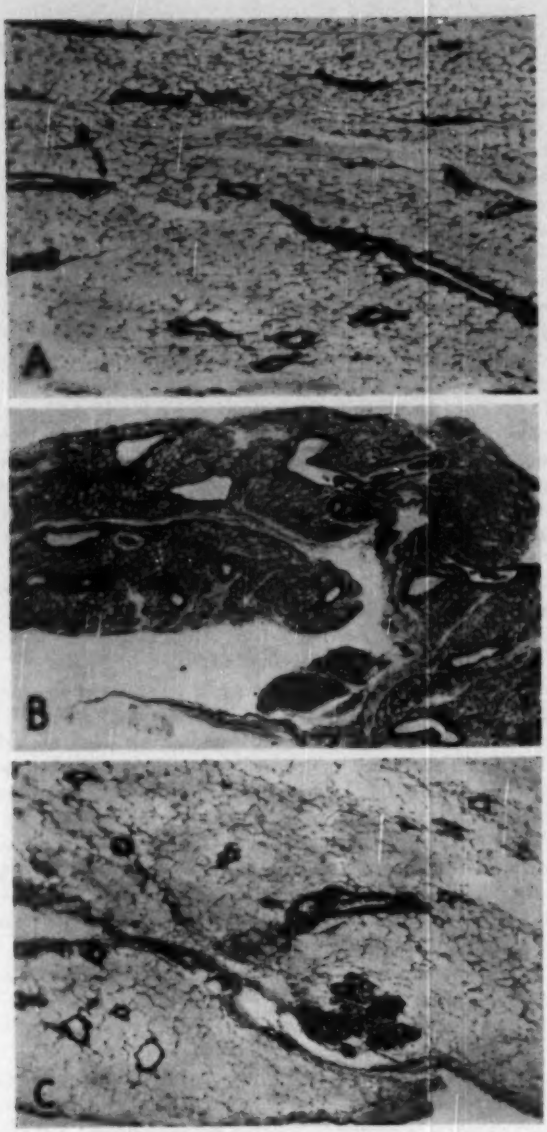

Fig. 2.—Each mouse received a graft of four ovaries at the age of 1 month: *A*, mouse 12 months old; the mammary gland shows abundant ductal proliferation (advanced effect). *B*, mouse 13½ months old; the mammary gland shows hypertrophy and intraductal hyperplasia (advanced effect). *C*, mouse 17 months old; the mammary gland shows ductal stimulation and alveolar growth (advanced effect).

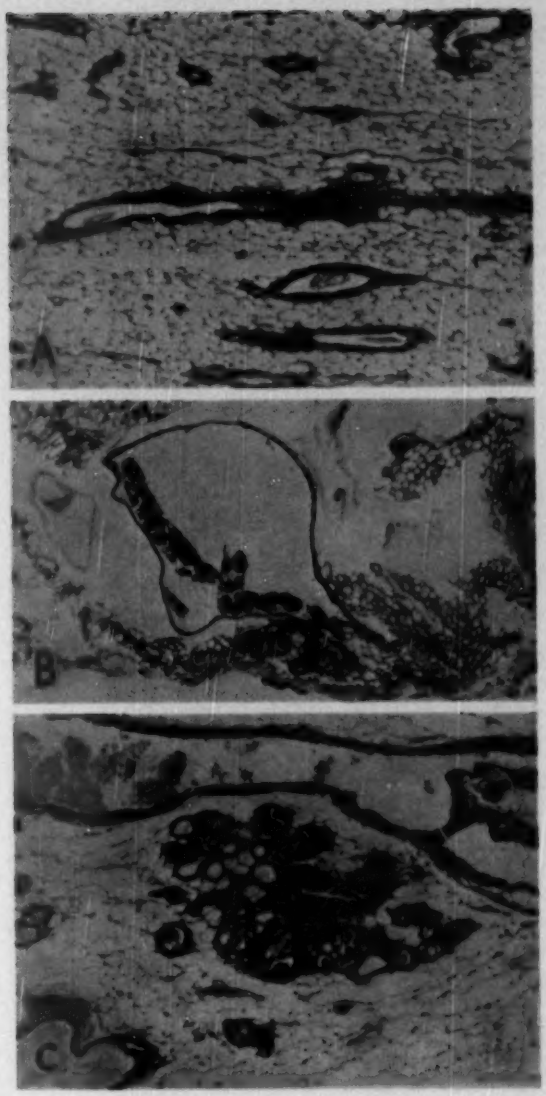

Fig. 3.—Each mouse received a combination graft of four anterior lobes of hypophyses and four ovaries at the age of 1 month. *A*, mouse 13 months old; the mammary gland shows widespread ductal growth and secretion with slight budding (advanced effect). *B*, mouse 19 month old; the mammary gland shows enlarged ducts with cyst formation and secretion and alveolar growth with secretion (advanced effect). *C*, mouse 7 months old; the mammary gland shows advanced ductal and alveolar hypertrophy and proliferation with secretion. In this animal a cancerous growth developed in another gland.

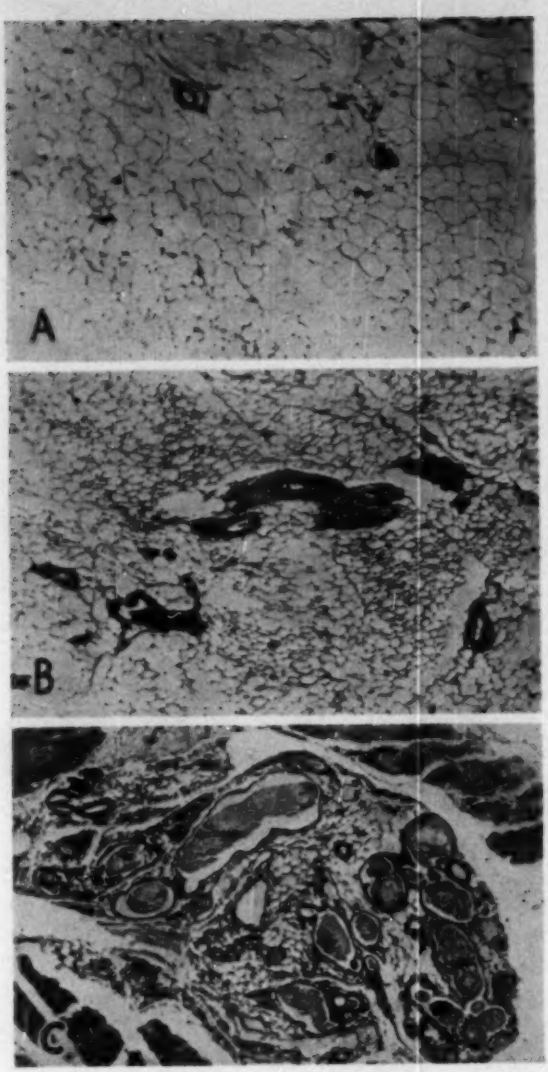

Fig. 4.—Each mouse received four anterior lobes of hypophyses at the age of 7 months. *A*, mouse 24 months old; the mammary gland is in a resting state (no effect). *B*, mouse 15 months old; the mammary gland shows slight ductal proliferation and secretion (slight effect). *C*, mouse 15 months old; the mammary gland shows striking ductal hypertrophy and secretion (advanced effect).

3. Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Anterior Hypophysial and Ovarian Transplants at the Age of 1 Month (26 Mice).—In 8 animals (30.8 per cent) mammary cancers were observed at the mean age of 12.9 months. The mammary glands of 25 mice were studied microscopically (fig. 3). The glands of 8 mice

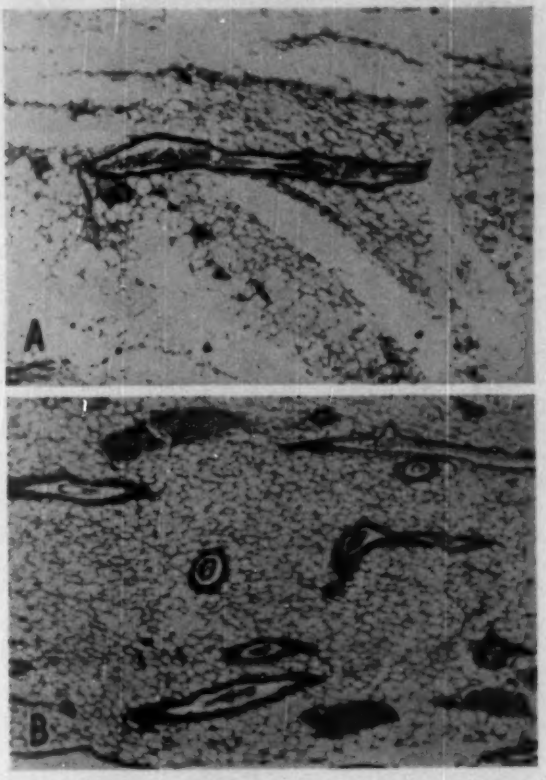

Fig. 5.—Each mouse received a graft of four ovaries at the age of 7 months. *A*, mouse 12 months old; the mammary gland shows slight ductal growth and hypertrophy with secretion (slight effect). *B*, mouse 13 months old; the mammary gland shows extensive ductal proliferation with secretion and slight budding (advanced effect).

(32 per cent) dying at a mean age of 18.5 months were resting, and those of 4 mice (16 per cent) dying at a mean age of 19.3 months showed slight ductal stimulation. The glands of the remaining 13 mice

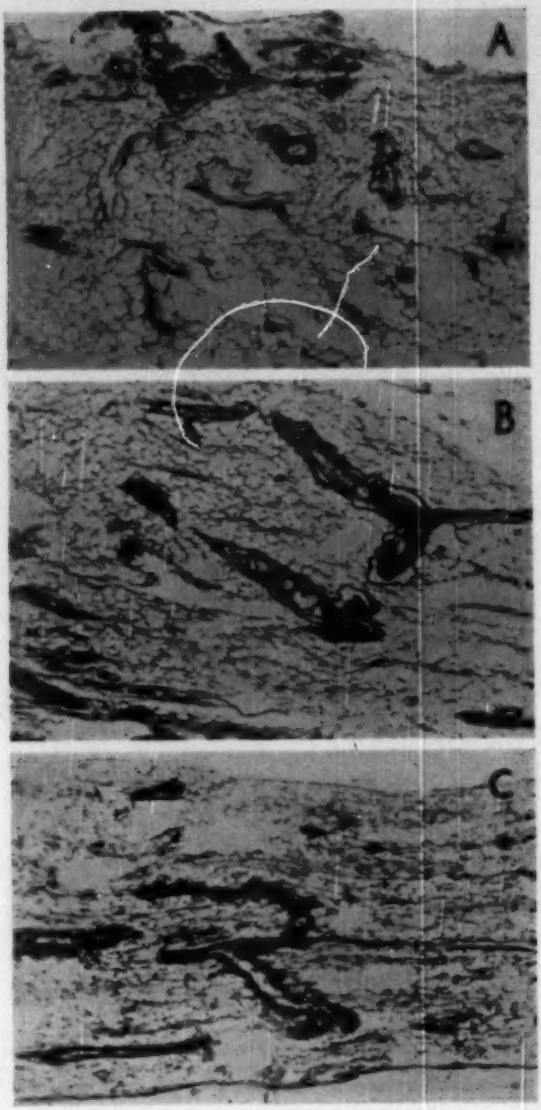

Fig. 6.—Each mouse received a combination graft of four anterior lobes of hypophyses and four ovaries at the age of 7 months. *A*, mouse 22 months old; the mammary gland shows slight ductal stimulation and secretion (slight effect). *B*, mouse 15 months old; the mammary gland shows slight ductal growth with hypertrophy and secretion (slight effect). *C*, mouse 14 months old; the mammary gland shows advanced stimulation of ductal growth with budding and slight alveolar growth (advanced effect).

(52 per cent), dead at 15.7 months of age, disclosed advanced ductal proliferation and secretion. In 12 of the latter animals, alveolar growth and secretion were likewise accentuated (fig. 3 *B* and *C*).

OLDER AGE GROUPS

4. *Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Anterior Hypophysial Transplants at the Age of 7 Months (28 Mice).*—Mammary cancers were not observed. The mammary glands of 21 mice were sectioned (fig. 4). In 15 of these mice (71.4 per cent), examined at the mean age of 21.2 months, the glands were found in a resting state. Four mice (19 per cent) dead at the mean age of 16.6 months showed slight ductal growth and secretion, and 2 animals (9.5 per cent) dead at the age of 15.0 months had advanced ductal proliferation and secretion (fig. 4 *C*); however, no alveolar growth had occurred.

5. *Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Ovarian Transplants at the Age of 7 Months (26 Mice).*—In none of these animals had cancerous transformation of the mammary tissue taken place. Microscopic examination of the mammary glands of 22 animals gave the following results (fig. 5): The glands of 18 animals (81.8 per cent) dying at the mean age of 17.6 months were resting. In 1 mouse (4.6 per cent) killed at the age of 17.0 months, slight ductal proliferation and secretion were noted. The mammary glands of 3 mice (13.6 per cent) examined at the mean age of 14.0 months showed advanced ductal growth and secretion; in all 3 animals alveolar growth and secretion were seen.

6. *Mice Orchidectomized at the Age of 3 to 4 Weeks and Receiving Anterior Hypophysial and Ovarian Transplants at the Age of 7 Months (29 Mice).*—No mammary cancers had developed. The mammary glands of 20 animals were examined microscopically (fig. 6). The glands of 12 mice (60 per cent) dead at the mean age of 15.6 months were inactive. In an additional 3 mice (15 per cent) with a mean age of 17.2 months there was slight ductal proliferation with secretion, and in the remaining 5 mice (25 per cent) examined at a mean age of 15.6 months advanced ductal growth and secretion were seen. In 2 of these 5 mice the ductal stimulation was associated with alveolar growth and secretion.

FATE OF TRANSPLANTS

As a rule, no difficulties were encountered in recovering ovarian grafts if they were preserved at the time of necropsy. In a number of cases, however, in which hypophysial grafts or vascularized remnants of hypophysial tissues were thought to be present at gross inspection, subsequent microscopic examination failed to substantiate the gross

impression; instead of the transplant, small fibrous nodules, small nodules of mammary gland tissue or tiny lymph nodes were seen in the section. Slight variations in the degree of relationship between the recipient and the donor, in the age of the donor or of the recipient at the time of transplantation, injury to the tissue to be transplanted or such local factors as vascular supply or lymphocytic reaction in the vicinity of the graft may account for the failure of syngenesiotransplants to take as well as for variations in the time of their survival.[4] It must be assumed, therefore, that in our experiments some grafts underwent atrophy or resorption or fibrous tissue replacement. On the other hand, small fragments of hypophysial transplants were found in the fat tissue of sectioned mammary glands where nothing had been noted at gross examination. Few grafts showed no encapsulation; usually the transplants were surrounded by varying amounts of connective tissue, and some glands had undergone fibrosis. Presumably, atrophy and fibrous replacement may lead to disappearance of some grafts, whereas others may survive for indefinite periods (fig. 7 *A* and *B*). Differences in the resistance of the various constituents of the ovary to transplantation have been pointed out by Loeb.[4] Perfectly formed graafian follicles and regular corpora lutea were found as late as fourteen months after transplantation (fig. 7 *C* and *D*). In many instances, however, fibrosis resulted in partial or complete obliteration of the typical structures. In view of the conflicting concepts of the influence of estrogen and particularly of that of progesterone on mammary growth, it would have been interesting to correlate the amount of follicular or interstitial gland tissue found in the grafted ovaries with the degree of stimulation of mammary growth. However, the present experiments were not designed for an investigation of this problem, and from the material available no valid conclusions seemed possible.

The conditions of the transplants at necropsy, the percentage of "takes" and the response made by the mammary gland to hormonal stimulation are recorded in table 2.

As seen from table 2, fewer grafts survived in the older than in the younger age groups: In 35.6 per cent of the younger and in 23.8 per cent of the older mice preserved anterior hypophysial transplants were detected. Of ovaries grafted in the younger group, 38.5 per cent were found to survive, compared with 31.8 per cent in the older group. Finally, of the mice receiving anterior lobes of hypophyses and ovaries, surviving grafts were present in 64 per cent of the younger and 35 per cent of the older group. The relatively low survival rate of the grafts in the older age group was even more striking in view of the fact that these grafts had remained in the host for a shorter period than the grafts made at the early ages.

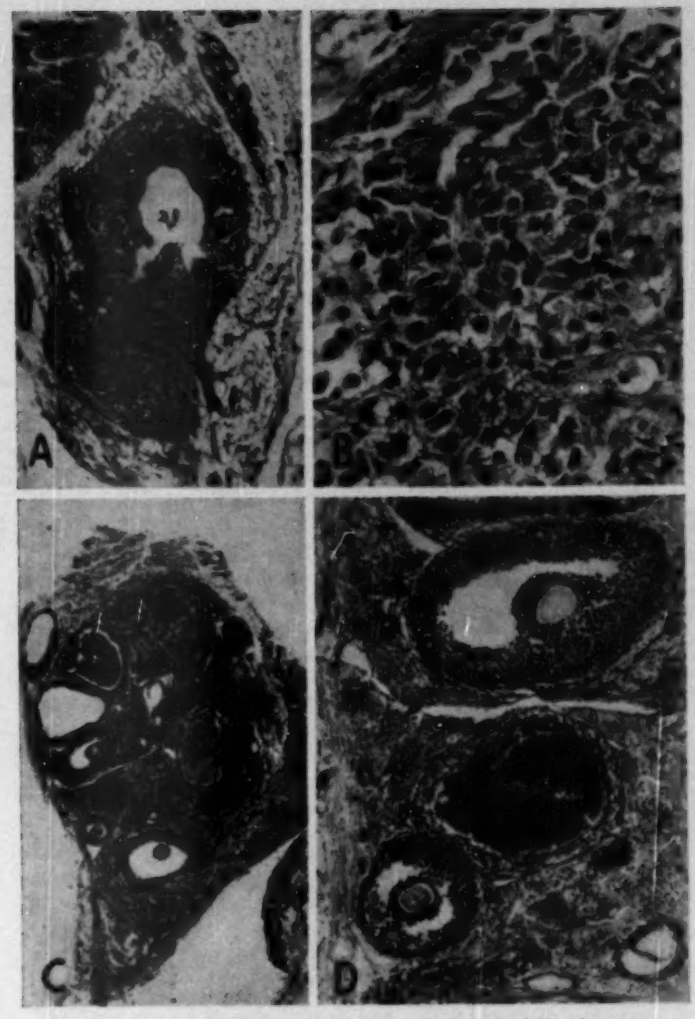

Fig. 7.—*A*, section through a preserved anterior hypophysial graft twenty-one months after transplantation. The graft is surrounded by connective tissue, and a cyst has formed in the center. Magnification, × 170.

B, an area of *A* under higher magnification (× 345). The hypophysial tissue is well preserved.

C, section through a preserved graft of an ovary fourteen months after transplantation. The graft is surrounded by connective tissue and is well preserved, showing follicles in various stages of maturation. Magnification, × 25.

D, an area of *C* under higher magnification (× 93). The follicles are well preserved and functioning, with formation of corpora hemorrhagica.

RESULTS OF CONTINUING SURVIVAL AND OF DISAPPEARANCE OF GRAFTS

In regard to the relation of the growth response of the mammary gland and the state of preservation of the graft one may distinguish four combinations of results: (1) mammary glands stimulated in the presence of surviving grafts; (2) mammary glands resting in the presence of surviving grafts; (3) mammary glands stimulated in the absence of surviving grafts; (4) mammary glands resting in the absence of surviving grafts.

Stimulation of Mammary Growth in the Presence of Surviving Grafts.—In the younger group this result was observed in 4 of 6 mice bearing anterior hypophysial, in 9 of 10 bearing ovarian, and in 12 of

TABLE 2.—*Fate of the Transplants and the Conditions in the Noncancerous Mammary Glands*

Exper. Group	Glands Transplanted to Mice Castrated at 2 Weeks	Mice Receiving Grafts	Mice from Which Grafts Were Recovered		No. Showing Indicated Microscopic Condition of Mammary Glands			Mice in Which Grafts Were Questionably Present or Absent		No. Showing Indicated Microscopic Condition of Mammary Glands		
			No.	% of Total	Resting	Slight Stimulation	Advanced Stimulation	No.	% of Total	Resting	Slight Stimulation	Advanced Stimulation
1	Ant. hypophysial lobes at 1 mo.	17	6	35.6	2	3	1*	11	64.4	7	2	2
3	Ovaries at 1 mo.	26	10	38.5	1	4	5†	16	61.5	5	3	8‡
2	Ant. hypophysial lobes and ovaries at 1 mo.	25	16	64.0	4	3	9‡	9	36.0	4	1	4†
4	Ant. hypophysial lobes at 7 mo.	21	5	23.8	1	1	3	16	76.2	14	2	0
5	Ovaries at 7 mo.	22	7	31.8	4	0	3‖	15	68.2	14	1	0
6	Ant. hypophysial lobes and ovaries at 7 mo.	20	7	35.0	3	1	3¶	13	65.0	9	2	2

* Alveolar growth was present.
† Four animals showed alveolar growth.
‡ Eight animals showed alveolar growth.
‖ Two animals showed alveolar growth.
¶ Three animals showed alveolar growth.

16 bearing both anterior hypophysial and ovarian grafts; in the older group 4 of 5 animals bearing anterior hypophysial, 3 of 7 bearing ovarian, and 4 of 7 mice bearing both anterior hypophysial and ovarian grafts belonged in this category. The degree of relationship of host and donor apparently did not exert a decisive influence on the response of the mammary gland, since mammary growth was observed in recipients not closely related to the donors. This was probably due to the fact that the A strain has been closely inbred for so long that the degree of homozygosity attained even among not closely related individuals is high enough to permit grafts to take and to function.

Resting Mammary Glands in the Presence of Surviving Grafts.—In the younger age group this was seen in 2 of 6 mice bearing anterior

hypophysial, in 1 of 10 bearing ovarian, and in 4 of 16 bearing both anterior hypophysial and ovarian grafts; in the older group 1 of 5 mice bearing anterior hypophysial, 4 of 7 mice bearing ovarian, and 3 of 7 bearing both anterior hypophysial and ovarian grafts showed no mammary stimulation in spite of the presence of living transplants. There is no reason to assume that these surviving grafts were not functioning. The mammary tissue of these individuals was probably comparatively nonsusceptible to the stimulating action of the hormones given off by the transplants.

Stimulation of Mammary Growth in the Absence of Surviving Grafts.—In the younger group this observation applied to 4 of 11 recipients of anterior hypophysial, to 8 of 16 recipients of ovarian, and to 5 of 9 recipients of both anterior hypophysial and ovarian grafts; in the older group 2 of 16 mice which had received anterior hypophysial, 1 of 15 which had received ovarian, and 2 of 13 which had received both anterior hypophysial and ovarian grafts showed proliferation of the mammary gland. These findings indicate that the grafts had functioned for a certain length of time before they underwent resorption or some other regressive change.

Resting Mammary Glands in the Absence of Surviving Grafts.—In the younger group this combination of findings was present in 7 of 11 recipients of anterior hypophysial, in 8 of 16 recipients of ovarian, and in 4 of 9 recipients of both anterior hypophysial and ovarian grafts; in the older group 14 of 16 mice bearing originally anterior hypophysial, 14 of 15 originally bearing ovarian, and in 9 of 13 originally bearing both anterior hypophysial and ovarian grafts had to be thus classified. In regard to these animals the question may be raised whether the transplants had taken at all or whether they had been resorbed before they could exert a stimulating effect or whether regression of previously stimulated mammary tissue had occurred.

It is unlikely that a previously stimulated mammary gland returned to a resting state without leaving evidence of stimulation such as persistence of small nodules or collapse-fibrosis.[5] In deciding whether or not functional grafts had been present at one time, the leukemia which occurred in some of the animals that did not show changes in the mammary gland may be helpful. In all likelihood that leukemia would not have developed (spontaneous incidence, 3.8 per cent)[6] unless a stimulus had been active for some time at least. If leukemia was present, the lack of response of the mammary tissue must be attributed to conditions within the mammary gland itself and not to a failure of the graft

5. Gardner, W. U.; Strong, L. C., and Smith, G. M.: Am. J. Cancer **37**: 510, 1939. Silberberg and Silberberg.[14, 8]

6. Kirschbaum, A., and Kaplan, H. S.: Science **100**:360, 1944.

to take or to function. As for the early fate of the grafts in those mice that showed stimulation neither of the mammary gland nor of the hemopoietic tissue, it remains unknown.

COMPARISON OF EXPERIMENTAL GROUPS WITH RESPECT TO SIMULTANEOUS STIMULATION OF MAMMARY AND HEMOPOIETIC TISSUES

In table 3 the incidence of simultaneous stimulation of mammary gland and hemopoietic tissues in the various experimental groups is shown. The data on leukemic changes are taken from a previous publication.[14] Only those animals are included in which both mammary

TABLE 3.—*Frequency of Simultaneous Growth Stimulation of Mammary Glands and Hemopoietic Tissues in the Various Experimental Groups*

Exper. Group	Glands Transplanted to Mice Castrated at 3 Weeks	Mice in Which Tissues Were Examined Microscopically	Mice Showing Indicated Conditions of Mammary Glands and Hemopoietic Tissues									
			Mammary Cancer + Leukemia +		Proliferation of Mammary Gland + Leukemia +		Proliferation of Mammary Gland + Leukemia —		Proliferation of Mammary Gland — Leukemia +		Proliferation of Mammary Gland — Leukemia —	
			No.	%	No.	%	No.	%	No.	%	No.	%
1	Ant. hypophysial lobes at 1 mo...	17	0	0	2	11.8	5	29.4	6	35.3	4	23.5
2	Ovaries at 1 mo.....	25	0	0	3	12.0	13	52.0	7	28.0	2	8.0
3	Ant. hypophysial lobes and ovaries at 1 mo.....	24	2	8.3	7	29.2	7	29.2	2	8.3	6	25.0
	Total.....	66	2	..	12	18.2	25	37.9	15	22.7	12	18.2
4	Ant. hypophysial lobes at 7 mo...	21	0	0	2	9.5	4	19.0	4	19.0	11	52.5
5	Ovaries at 7 mo.....	22	0	0	0	0	4	18.2	6	27.3	12	54.5
6	Ant. hypophysial lobes and ovaries at 7 mo.....	20	0	0	3	15.0	5	25.0	3	15.0	9	45.0
	Total.....	63	0	0	5	7.9	13	20.6	13	20.6	32	50.8

tissue and the internal organs were used for microscopic examination. In 2 (8.3 per cent) of 24 animals of the younger group carrying both anterior hypophysial and ovarian grafts, there was coexistence of leukemia and mammary cancer. This combination was not found in any other experimental group. Of 66 mice of the younger group, 12 animals (18.2 per cent) showed varying degrees of noncancerous hyperplasia of the mammary tissue associated with leukemic change. In the older group this combination was obtained in 5 of 63 animals (7.9 per cent). In the younger group 12 animals (18.2 per cent) failed to disclose either leukemic changes or proliferation of the mammary glands; in the older group there were 32 mice (50.8 per cent) with no reaction in either tissue. Stimulated mammary growth without leukemic change was noted in 25 animals (37.9 per cent) of the younger and in 13 animals (20.6 per cent) of the older group. Finally, leukemic changes in association with resting of the mammary glands were

observed in 15 animals (22.7 per cent) of the younger and in 13 animals (20.6 per cent) of the older group. There is thus no good correlation between these two types of growth, and these results would support the view that the mechanisms responsible for stimulation of the growth of mammary and hemopoietic tissues, respectively, are not identical.

COMMENT

In orchidectomized mice of strain A, syngenesiotransplants of anterior lobes of hypophyses, of ovaries or of both anterior lobes and ovaries are able to induce proliferation, hypertrophy and secretion in the mammary gland.

Irrespective of the type of experiment, mammary growth was more activated if the grafts were alive at the time of necropsy than if they had perished. Correspondingly, more resting mammary glands were found in the absence of living grafts than in those mice in which the grafts had persisted.

The effects exerted by the hypophysial grafts manifested themselves primarily in hypertrophy and increased secretion of the ductal epithelium; in addition, there were elongation of old and budding of new ducts, and occasional slight stimulation of alveolar growth. Intensification of mammary growth and secretion following repeated or intermittent administration of anterior hypophysial substances has been observed by a number of investigators. The problems involved have been reviewed from time to time.[7] Yet considerable differences exist as to the interpretation of the results and as to the manner in which the hypophysis acts on the mammary gland. In our castrate mice the stimulation of mammary growth and secretion must be independent of the sex glands and may be attributed to some substance or substances given off by the hypophysial grafts. These secretions may act directly on the mammary tissue and may possibly be under the influence of the adrenal glands. The latter mechanism of mammary carcinogenesis has recently been emphasized because mammary cancers have occurred in mice of certain strains and in various hybrids which were castrated soon after birth.[8] In these animals adenoma developed in the adrenal cortex and hyperplastic basophilic nodules in the anterior lobes of the hypophysis; moreover, in the mammary tissue hyperplastic changes and cancers were observed. Hypophysial hormones were considered to be the direct cause of this mammary growth. Evidence is accumulating that estrogenic hormone is not necessarily involved in all forms of

7. Riddle, O.: Ann. Rev. Physiol. **3**:573, 1941. Mixner, J. P., and Turner, C. W.: Research Bulletin 378, University of Missouri, College of Agriculture, Agricultural Experiment Station, 1943. Bittner, J. J.: Cancer Research **8**:625, 1948. Loeb.[1c] Loeb.[2a]

8. Dickie, M. M., and Woolley, G. W.: Cancer Research **9**:372, 1949.

mammary carcinogenesis. In an as yet unpublished series of experiments, mammary cancer developed in a female mouse of strain A which was ovariectomized at the age of 1 month and was receiving transplants of the anterior parts of the hypophysis. The urteus and vagina of this animal showed advanced atrophy, a condition that is not very well compatible with the presence of estrogenic hormone. Moreover, adrenal cortical adenomas failed to show estrogenic activity in ovariectomized mice of strain A.[9]

The slight alveolar growth observed in our male castrates after they had received the hypophysial transplants was seen only in association with marked ductal proliferation; on the other hand, low grade ductal stimulation was not accompanied by alveolar growth. It thus seems that alveolar growth represents an accentuation and extension of ductal growth rather than a process of fundamentally different nature. If two different growth stimuli were active, corresponding to two different hormones given off by the anterior hypophysial grafts, one would expect to find, here and there at least, mammary glands in which both slight ductal proliferation and alveolar growth were present.

After transplantation of ovaries, fewer mice showed resting mammary tissue, and more animals showed advanced ductal and alveolar stimulation, than after transplantation of anterior lobes of hypophyses. Still, in one respect the effect of ovarian transplants resembled that produced by the hypophysial grafts. In the animals in which the ducts were only slightly or moderately stimulated, no alveolar proliferation was noted; correspondingly, alveolar growth did not occur without simultaneous vigorous growth of the ducts. While this observation does not rule out separate roles of estrogen and progesterone in mammary growth, it may be interpreted as a cumulative stimulating effect of estrogen and progesterone produced by the ovarian grafts. Alveolar growth would then again simply constitute an intensification of ductal proliferation, occurring, if the stimulus is sufficiently powerful, independently of the nature of the stimulus and the mechanism by which it acts.

Within each age group, the most intense mammary growth was obtained by combined transplantation of anterior lobes of hypophyses and ovaries. This advantage of combined transplantation becomes particularly striking if the animals showing advanced mammary stimulation are considered separately from those in which mammary growth was slight. The accentuation of mammary growth observed after combined grafting of anterior lobes of hypophyses and ovaries may represent a cumulative effect of the stimulation exerted by these two types of transplants. In addition, however, the transplanted ovaries may themselves be stimulated by gonadotropin given off by the grafted

9. Frantz, M. J., and Kirschbaum, A.: Cancer Research **9**:596, 1949.

anterior lobes. To such gonadotropic activity and subsequent increased output of estrogen of the animal's own ovaries Loeb and Kirtz [10] attributed the increased incidence of mammary cancers in virgin female mice carrying anterior hypophysial grafts. The transplanted anterior lobes of hypophyses may perhaps act similarly on grafted ovaries: The mammary glands of the recipients would thus be subject not only to a direct influence of the transplants but also to an indirect one, namely, an increased output of the estrogenic hormone of the grafted ovaries.

In animals of the older age groups, mammary growth was less stimulated than in the mice of the younger series. Accordingly, the percentage with resting glands was as a rule higher in the older than in the corresponding younger groups. This applies to mice in which the grafts persisted as well as to those in which they perished, although there had been fewer "takes" in the animals of the older than in those of the younger age groups. Only in the mice receiving hypophysial grafts and showing surviving transplants was an exception to this rule noted. However, the number of animals available—2 of 6 in the younger, and 1 of 5 in the older age group—seems too small to permit conclusions as to the significance of these findings.

The results of the microscopic examination of the noncancerous mammary glands paralleled closely the incidence of mammary cancers in the present series. Conditions most favorable to the production of mammary cancer were early age at transplantation and grafting of ovaries together with anterior lobes of hypophyses. The same conditions were likewise most conducive to the production of hyperplastic mammary growth.

SUMMARY

In male mice of strain A castrated at the age of 3 to 4 weeks, syngenesiotransplants of anterior lobes of hypophyses, of ovaries or of a combination of the two types of glands stimulate cancerous and noncancerous growth of the mammary glands in the order mentioned. The effectiveness of these grafts in relation to their survival has been discussed. Although stimulation of alveolar growth may be caused by all three kinds of grafts, it is most common after the combined transplantation of anterior lobes of hypophyses and ovaries. Alveolar growth is considered to be the result of an accentuation of ductal proliferation. Grafts made in 1 month old castrates cause more marked stimulation of mammary growth than similar grafts made in 7 month old castrates.

The increased growth processes of the mammary gland and the hemopoietic tissues showed no correlation in the various experimental groups, and the coexistence of mammary cancer and leukemia observed in 2 mice was considered to be a chance phenomenon. Two different sets of factors are therefore thought to be involved in mammary carcinogenesis and leukemogenesis, respectively, under the conditions of the present experiments.

Notes and News

Fellowships in Exfoliative Cytology.—The American Cancer Society announces that 31 fellowships in exfoliative cytology are available for 1950. Each fellowship, amounting to $140 per month, will be awarded for a period of four months and is not renewable. Physicians who are graduates of class A medical schools, have completed two years of postgraduate training in pathology and are otherwise qualified may apply directly to the following directors of laboratories where the fellowships are available:

Dr. George N. Papanicolaou
Cornell University Medical College
New York

Dr. Otto Saphir
Michael Reese Hospital
Chicago

Dr. Lewis C. Scheffey
Jefferson Hospital
Philadelphia

Dr. Ralph E. Kendall
Hartford Hospital
Hartford, Conn.

Dr. Locke L. Mackenzie
New York Post-Graduate Hospital
New York

Dr. Samuel C. Harvey
Yale University School of Medicine
New Haven, Conn.

Dr. Warren C. Hunter
University of Oregon Medical School
Portland, Ore.

Dr. Lauren V. Ackermann
Washington University School of Med.
St. Louis

Dr. Herbert F. Traut
University of California Hospital
San Francisco

Dr. Arthur T. Hertig
Free Hospital for Women
Brookline, Mass.

Dr. John R. McDonald
Mayo Clinic
Rochester, Minn.

NOTICE

The index for Volume 49 will be mailed with the July 1950 number.

Books Received

INTRODUCTION TO NEUROPATHOLOGY. By Samuel Pendleton Hicks, M.D., department of pathology of the Harvard Medical School and the New England Deaconess Hospital (formerly consultant in neuropathology, National Naval Medical Center); and Shields Warren, M.D., departments of pathology of the Harvard Medical School and the New England Deaconess Hospital; director of the division of biology and medicine, United States Atomic Energy Commission. First edition. Pp. 494, with 289 illustrations. Price $10. McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 18; Aldwych House, Aldwych, London, W.C. 2 London; Toronto, 1950.

Too long have general pathology and neuropathology been merely nodding acquaintances. Most neuropathologists have had relatively little fundamental pathologic training, and, conversely, most general pathologists feel lost in the field of brain disease. This new volume by Hicks and Warren is an attempt to bridge the gap, and to secure a closer correlation between general pathologists and specialists in the field of nervous and mental disease. It is particularly addressed to "medical students and to trainees in the neurologic specialties and pathology." A physiologic approach has been largely utilized.

In its purpose the book is only partially successful. The chapters exemplify a conventional subdivision of the subject: reaction patterns; circulatory disturbances; disturbances of cerebrospinal fluid and pressure relations; inflammatory diseases; metabolic, toxic and degenerative diseases; mechanical injuries; tumors, and maldevelopments, mental disease and epilepsy. The chapters are quite uneven. By far the best is the treatment of tumors, in which, of course, the authors have had extensive experience. Excellent is the résumé on brain metabolism. Circulatory disorders and vascular accidents, a field in which the interested general pathologist finds abundant material, are well handled.

The volume is an excellent work of book-making, with abundant illustrations splendidly reproduced. As an introductory work, well suited to medical students, this text can be recommended, for it contains considerably more detail than is found in textbooks of general pathology. Furthermore, it is well supplied with current bibliographic references, a refreshing number of which are from non-neurologic journals.

However, for the student at the resident level, either in neurologic specialties or in pathology, and for the mature neurologist or pathologist, the usefulness of the book is limited. The more specific neurologic conditions, representing diseases with which the general pathologist has relatively little contact, are not so adequately covered. For example, parkinsonism receives only two lines. The whole group of cerebellar atrophies and Friedreich's ataxia receives only twelve lines. The discussion of Alzheimer's disease may be particularly criticized for misleading and unproved statements and for overwhelming reliance on work subsequently shown to be due to technical artefacts. The whole subject of the demyelinating diseases appears inadequately treated. The discussion of reaction patterns is replete with statements that can be challenged, such as the claim that corpora amylacea form in astrocyte cytoplasm, and that chromatolysis can be the result of postmortem autolysis. There are some inconsistencies; for example, chromatolysis is considered as cloudy swelling on one page and as a chronic atrophic change on another.

The coverage of the literature is neither wide nor deep, and there is no effort to present the most fundamental or most complete references. Out of the whole field of neuropathology only two German references are cited in the entire book.

Virtually all the photomicrographs are from hematoxylin and eosin or phosphotungstatic acid–hematoxylin stains, with a few silver and myelin preparations.

So far as can be ascertained by this reviewer, all sections are paraffin, and no celloidin preparations were employed. Not a single Nissl stain is illustrated. The work suffers from not having any low power photomicrographs of general structure.

The text succeeds in synthesizing general and nervous system pathology at an introductory level. But for detailed coverage, or for discussion of controversial points, it requires supplementation such as can be found in current texts specifically neuropathologic in scope.

CHEMOTHERAPY OF LEUKEMIA AND LEUKOSARCOMA. By William Dameshek, M.D.; Marvin L. Bloom, M.D.; Louis Weisfuse, M.D.; Milton H. Freedman, M.D., and Miguel Layrisse, M.D., New England Center Hospital, Tufts College Medical School, Boston. Pp. 53. Price $4.75. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1950.

This 54 page loose leaf book is a reproduction of an exhibit presented at the American Medical Association Convention in Atlantic City, N. J., in June 1949. It contains charts on the classification, the causation, the diagnostic features and the hematologic and cytologic studies of leukemia and leukosarcoma. The pharmacology, indications, limitations and toxic effects of chemotherapy are given for such agents as the nitrogen mustards, the folic acid antagonists and urethane. There are ten pages on Hodgkin's disease, in which nitrogen mustard has a certain degree of specificity, and three pages on multiple myeloma, in which urethane offers the most satisfactory form of therapy to date. The book contains a number of instructive tables and diagrams. Most of the illustrations are good. Cases illustrative of treatments are presented in a concise and instructive manner. Although the subject matter of this book is common knowledge to most clinicians, pathologists and hematologists, the format of the book should prove useful for didactic purposes.

HANDBOOK OF MEDICAL MANAGEMENT. By Milton Chatton, M.D., instructor in medicine, University of California Medical School, San Francisco; Sheldon Margen, M.D., clinical instructor in medicine and research associate in medicine, University of California Medical School, San Francisco; and Henry D. Brainerd, M.D., assistant clinical professor of medicine and pediatrics, University of California Medical School, San Francisco; assistant clinical professor of pediatrics, Stanford University School of Medicine; physician in charge, isolation division, San Francisco Hospital. Pp. 476. Price $3. University Medical Publishers, Postoffice Box 761, Palo Alto, Calif., 1949.

This recently published, pocket size handbook is a concise guide to the medical management of disease. It is a valuable reference to those who seek accurate chemical and physiologic data, of which the authors have assembled an imposing volume. An attempt is made toward simplified definition and diagnosis of pathologic entities.

The addition of code numbers of diseases from the "Standard Nomenclature of Disease and Standard Nomenclature of Operations" of the American Medical Association is to be commended. The proper classification of records will thus be facilitated.

Some difficulty was experienced in locating specific information, owing to the lack of a comprehensive table of contents and index.

The book undoubtedly fills the need for a source reference and should be warmly welcomed by intern, resident and practitioner.

CHEMICAL DEVELOPMENTS IN THYROIDOLOGY. By William T. Salter, M.D., professor of pharmacology, Yale University School of Medicine, New Haven, Conn. Pp. 87. Price $2. Charles C Thomas, 220 E. Monroe St., Springfield, Ill., 1950.

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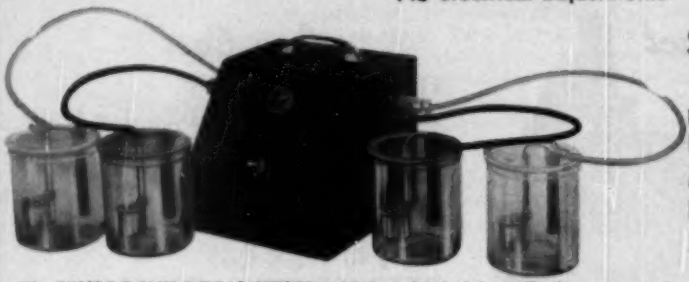

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